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Communications

Organic Reactive Intermediates: Part XI—Preparation & Reactions of Xanthenyl Ylids

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Received 19 *October* 1987; *accepted* 4 *December* 1987

Two new xanthenyl ylids, viz. triphenylphosphoniumxanthen-9-ylid and triphenylphosphoniumthioxanthen-9-ylid have been prepared. The former undergoes normal Wittig reaction with a number of carbonyl compounds yielding olefins.

No report exists in the literature about the ylids derived from xanthene or its thio analogue. In principle such ylids should exist and it would be of interest to speculate on a host of synthetically important reactions that these ylids should undergo. As a preliminary step in this direction, we report in this communication, the preparation of two new xanthenylids, viz. triphenylphosphoniumxanthen-9-ylid (**4b**) and the results of their Wittig reactions under mild conditions with carbonyl compounds.

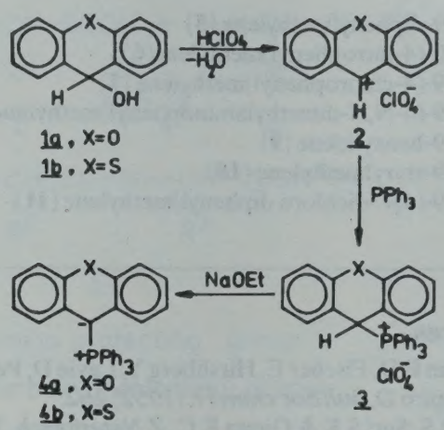
The ylids **4a** and **4b** were isolated as a red solid, m.p. 97-99° and an orange red solid, m.p. 129° respectively, following a simple synthetic strategy outlined in Scheme 1.

The general procedure for preparing the phosphonium salts (**3**) is typified by the following preparation of triphenylphosphoniumxanthen-9-yl perchlorate (**3a**).

A nitromethane solution (40 ml) of triphenylphosphine (10 g, 38.2 mmol) was treated with xanthylium perchlorate (**2a**), obtained *in situ* by adding $HClO_4$ (10%, 4 ml) dropwise to a stirred solution of 10% xanthydrol (**1a**) in methanol (79.0 ml, 40 mmol). The mixture was warmed to 60° and on allowing to stand at 15°, a crystalline mass of **3a** separated out, which was recrystallised from nitromethane-ethyl acetate to furnish a fine crop of colourless needles, m.p. 234°; yield 20.7 g (96%) (Found: C, 68.7; H, 4.3; O, 14.6; P, 5.2. $C_{31}H_{24}ClO_5P$ requires C, 68.6; H, 4.5; O, 14.7; P, 5.7%).

Analogously, triphenylphosphoniumthioxanthen-9-yl perchlorate (**3b**), m.p. 236-38° was prepared in around 93% yield.

For obtaining the ylid (**4a**), a suspension of **3a** (2.5 g, 4.6 mmol) in dry ether was taken and a little dry ethanol was added to it. The mixture was shaken with a small piece of sodium for 30 min, which resulted in the

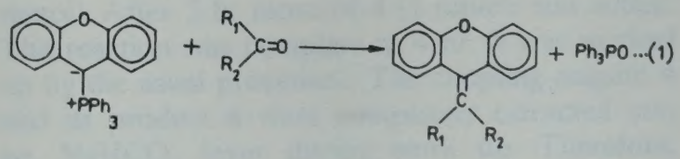


SCHEME 1

separation of **4a** as a red solid, m.p. 97-99°; yield 1.8 g (89%) (Found: C, 84.6; H, 5.1; O, 3.6; P, 6.8. $C_{31}H_{23}OP$ requires C, 84.1; H, 5.2; O, 3.6; P, 7.0%). Equivalent weight by perchloric acid titration, 443.05 (Calc. 442.49).

In an analogous manner, **4b** was isolated as an orange red solid in about 93% yield, m.p. 129° and characterized by equivalent weight determination and elemental analyses.

From the point of view of stability **4b** is quite stable while **4a** survives for a week when both are stored as solids under ordinary laboratory conditions. The condensation of **4a** and **4b** with several carbonyl compounds was studied. The results show that while **4a** undergoes a normal Wittig reaction (Eq. 1, Table 1), **4b** fails and rather tends to hydrolyse to triphenylphosphine oxide and thioxanthene.



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The Wittig reactions of **4a** were carried out by adding solid ylid (**4a**, 1.8 mmol) to a stirred solution of carbonyl compound (1.8 mmol) in chloroform (30 ml). The mixture was stirred for 15 min, the solvent removed and the residue chromatographed on silica gel. Elution with pet ether (40-60°) gave the desired olefin. All the products were characterised by spectroscopic methods and by comparing their melting points with those reported in the literature.

The authors wish to thank CSIR, New Delhi for the award of a senior research fellowship to one of them (AK).

Table 1—Products Obtained in the Reaction of **4a** with Carbonyl Compounds

Olefin	R_1	R_2	yield %	m.p. °C	(lit.m.p.) °C
Xanthene, 9-diphenylmethylene (**5**)	C_6H_5	C_6H_5	65	198-200	(196-97)[1]
Xanthene, 9-(4-nitrophenyl)methylene(**6**)	p-$NO_2.C_6H_4$	H	92	167	(165-67)[2]
Xanthene, 9-(4-chlorophenyl)methylene (**7**)	p—$Cl.C_6H_4$	H	90	134	(134[3]; 137[4])
Xanthene, 9-(4-N,N-dimethylaminophenyl)methylene (**8**)	p-$NMe_2.C_6H_4$	H	83	123-25	—
Xanthene, 9-benzylidene (**9**)	C_6H_5	H	76	116	(116)[4]
Xanthene, 9-styrylmethylene (**10**)	$C_6H_5.CH=CH$	H	80	92	(91-92)[5]
Xanthene, 9-(4,4′-dichloro diphenyl)methylene (**11**)	p—Cl—C_6H_4	p—Cl—C_6H_4	79	208	(209-10)[5]

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N,N′-Bis(2-oxo-3-oxazolidinyl)phosphorodiamidic Azide: A Useful Coupling Agent in Peptide Synthesis†

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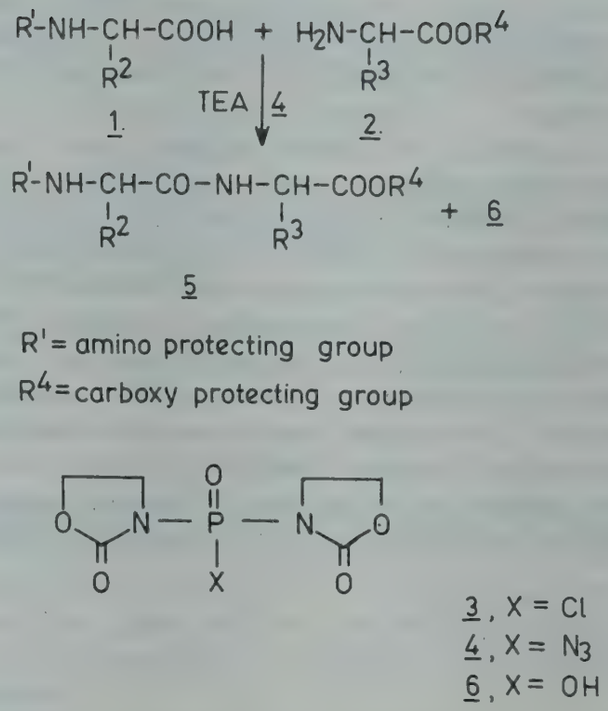
N,N′-Bis(2-oxo-3-oxazolidinyl)phosphorodiamidic azide (BOPA), has been developed as an efficient coupling reagent in peptide synthesis. Coupling reaction proceeds smoothly with no detectable racemization.

The crux of peptide synthesis lies in the activation of carboxyl component followed by aminolysis for generating the peptide bond. This has been achieved with a variety of coupling agents[1]. However, there are problems particularly in the synthesis of biologically active peptides containing unusual linkages besides the normal peptide bond[2,3]. This has necessitated the need for developing more versatile coupling agent(s) which would meet the new challenges. In this communication we present interesting observations on N,N′-bis(2-oxo-3-oxazolidinyl)phosphorodiamidic azide (BOPA, **4**) as a new coupling reagent in peptide synthesis.

Palomo-Coll and coworkers[4] have reported the synthesis and use of N,N′-bis(2-oxo-3-oxazolidinyl)phosphorodiamidic chloride (BOPDC, **3**) for making esters and amides of carboxylic acids. Recently BOPDC has been used for forming N-alkyl peptide bond with little racemization[5]. However, **3** suffers from certain drawbacks, viz. it is extremely hygroscopic and coupling reaction takes as much as 16-20 hr for completion. Therefore, we focussed our attention on derivatizing BOPDC. Of the several derivatives tested, the azide (**4**), prepared by the procedure reported in the literature[4] was found to be the most satisfactory‡. Using **4**, a model dipeptide Z-Phe-Ala-OMe was prepared in excellent yield.

Comparative studies on racemization carried out with Z-Val-Val-OMe and Z-Phe-Leu-OMe model systems suggested that the extent of racemization is the same as that observed in the case of DCC/HOBT method[6]. Our results are similar to those recently reported by Auwera *et al.*[7]



Scheme-1

A typical coupling reaction using **4** is given in Scheme 1. Carboxylic acid **1** (1 mmol) was stirred with triethylamine (1 mmol) and **4** (1.1 mmol) at 0°C for 10 min in methylene chloride (10 ml). To this was added suitably protected amino component **2** (1 mmol) followed by triethylamine (1 mmol). After 2 hr more of **4** (1 mmol) was added. The reaction was complete in 4 hr. It was worked up by the usual procedure. The coupling reagent **4** and its product **6** were completely extracted into aq. $NaHCO_3$ layer during work up. Therefore, peptides could be crystallized directly from the organic phase without further purification. Some typical yields after recrystallizations were: Z-Phe-Ala-OMe 75%, Bzl—Val-Val-OMe 70%; Z-Val-Val-OBzl 65%; Z-Phe-Leu-OMe 65%; Z-Pro-Val-OMe 70%; Boc-Asn-Pro-OBzl 60%; Boc-Ala-MePhe-OMe 60%; Z-Phe-MeAla-OMe 60%; Boc-Gly-Lys(Z)-Val-OMe 65% and Boc-Gly-Lys(Z)-Phe-Ala-OMe 70%. We have also demonstrated the application of **4** in derivatizing carboxy functions of amino acids and peptides by synthesizing the ester Z-Phe-ONp and the amide Z-Val-NH-CH_2-C_6H_5 in almost quantitative yields employing general procedure described above.

†CDRI Communication No. 4147

‡Following esters (**3**, X = 1-hydroxybenzotriazolyl, 6-nitro-1-hydroxybenzotriazolyl and 2-hydroxypyridyl) were prepared employing conditions of Schotten-Baumann reaction. All the derivatives were characterized by FDMS in which appropriate molecular ion peaks were detected.

All the known compounds gave satisfactory elemental analyses, m.ps. and optical rotations comparable with the reported values. Some of the new derivatives have been well characterized by elemental analyses, m.ps, PMR and FDMS data. Coupling of tripeptide was done by 2 + 1 coupling while that of tetrapeptide it was done by 2 + 2 coupling.

In summary, the present coupling agent is less hygroscopic and more stable than BOPDC. It is stable for several months in a desiccator. The water solubility of the product **6** offers a distinct advantage in the work-up procedure vis-a-vis DCC/HOBT and other conventional methods. The coupling yields are very good with no side products even in the case of Asn and Pro containing peptides. This coupling reagent would be useful in derivatizing C-terminal residues of biologically active peptides for the purpose of structure-activity studies. Furthermore, **4** could be useful like DPPA in the synthesis of cyclic peptides[8]. Further work along this line is in progress.

We thank Dr M M Dhar for his keen interest and encouragement. We also thank RSIC staff for providing spectral data of all the compounds.

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Photoreactions of Dispiro{naphthalene-1,2′(1′*H*)-naphtho[2,1-*b*]pyran-3′,1″-naphthalene}-2(1*H*),2″(1″*H*)-dione & Similar Dispironaphthalenones—Novel Case of β − C − C Cleavage in Enones

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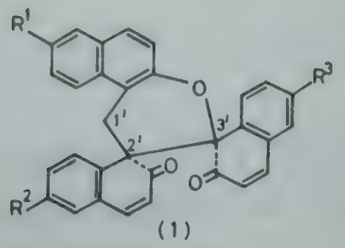
Irradiation of the dispironaphthalenones (**1**) affords wide range of products resulting from β-cleavages and intramolecular cycloaddition. These have been assigned structures (**2**), (**5**), (**6**), (**7**) and (**11**) on the basis of spectral data. Formation of these products has been rationalised.

Isolation of novel compounds, viz. *cis* and *trans* dispiro{naphthalene-1,2′(1′*H*)-naphtho[2,1-*b*]pyran-3′,1″-naphthalene}-2(1*H*),2″(1″*H*)-diones (**1a**) and (**2a**) along with known Abel's ketone (**3a**) and 1,2-naphthoquinone-1-methide dimer (**6a**), has been reported[1] in the oxidation of bis(2-hydroxy-1-naphthyl)methane with DDQ. The X-ray crystallographic investigation[1,2] of *cis*-**1a** revealed that the C(2′)-C(3′) bond is unusually long (1.616 Å) and that the two olefinic π-bonds of the enones are fairly well oriented for intramolecular cycloaddition. Consistent with the former observation, hydrogenation or zinc-acetic acid reduction of **1a** readily gave[1] the dihydroxy compound (**4a**) via cleavage of the C(2′)-C(3′) bond. It was, therefore, thought that **1a** could be a useful model for observing the less known photochemical β − C − C cleavage in enones as well as the intramolecular [π2S + π2S] cycloaddition.

Compound (**1a**) shows UV absorption maxima ($CHCl_3$) at 360 (ε 2,550), 348 (7,330), 320 (8,210), 312 (7,920), 294 (9,380), 280 (12,310), 268 (18,190), 262 (21,710) and 247 nm (30,210). The lowest excited state (S_1) of **1a** is estimated to be 332 kJmol^{-1}, based on absorption and emission spectra, and shows a highly structured emission in the region 360-520 nm in excitation to S_1 or S_2 states. No emission attributable to an intramolecular exciplex is seen although it has been observed in the case of compounds having parallel aromatic ring at optimum distances[3,4].

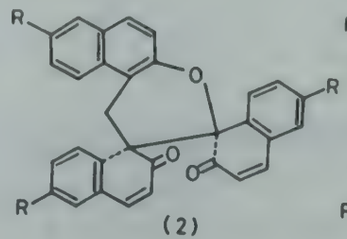
Irradiation of 0.01 *M* solution of **1a** in benzene usin 450 W medium pressure mercury vapour lamp has resulted in the formation of five products, designated as (A), (B), (C), (D), and (E). Based on spectral properties and elemental analyses, the dinaphthodioxin structure (**5a**) was assigned to compound (A) which was further confirmed by comparing with an authentic sample[5]. Compounds (B) and (D) were identified as the known 1,2-naphthoquinone-1-methide dimer (**6a**) and *trans*-dispironaphthalenone (**2a**) respectively by comparison with authentic samples[2,6].

PMR spectrum of compound (C) [$C_{31}H_{20}O_3$; IR (nujol): 1700 and 1680 cm^{-1}], isomeric with the starting (**1a**), exhibited an *ABq* centered at δ 2.74 ($\Delta\nu_{AB}$ = 81.0 Hz, J_{AB} = 14.3 Hz) indicating the presence of nonequivalent methylene protons and two pairs of mutually coupled vinylic protons (δ 5.47, 6.98 and 6.17, 7.54). Thus, the presence of two naphthalenone systems as in **1a** was evident. The other C_{10}-unit must be a fully aromatic naphthalene ring. Three structures **(7a)**, **(8)** and (**9**) are possible on the basis of above spectral data. As compound (**C**) exhibits an upfield *ABq* (δ 2.74; *cf.* **1a**, δ 3.79), structure (**7a**) containing nonbenzylic methylene protons, was preferred over the other two. Further, structures (**8**) and (**9**) could be ruled out in view of the large difference in the chemical shift values of the two spiro-carbon atoms (δ 55.00 and 82.46) in its CMR spectrum. In both the structures (**8**) and (**9**), the two spiro-carbon atoms having an α-oxygen should be deshielded and are expected to resonate at almost equal frequencies. Structure (**7a**) can lead to two stereoisomers, namely *cis* (*R,R*) and *trans* (*R,S*). The PMR of compound (C) displayed two well-shielded aromatic protons (δ 6.39 and 6.56). An inspection of the Drieding models showed that each of the isomers *cis* and *trans* can have two conformations, and out of the four possible conformations, only *cis*-**7a** with conformation as shown in Fig. 1 can explain the presence of two shielded aromatic protons (H_a and H_b); the other conformations will have only one shielded aromatic proton. Hence,



(1)

a) $R^1 = R^2 = R^3 = H$
b) $R^1 = R^2 = R^3 = Br$
c) $R^1 = Br, R^2 = R^3 = H$
d) $R^1 = R^3 = Br, R^2 = H$
e) $R^1 = R^2 = Br, R^3 = H$
f) $R^1 = R^2 = R^3 = Bu^t$
g) $R^1 = R^2 = R^3 =$



(2)

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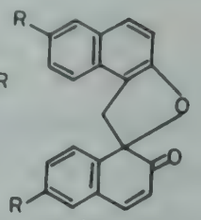
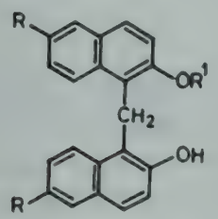
b) R = Br

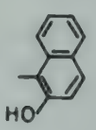
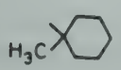


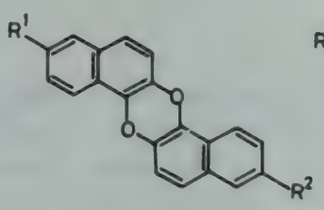
(3)

a) R = H
b) R =

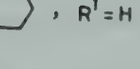


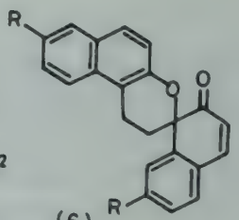
(4)

a) R = H, R^1 = 
b) R =  , R^1 = H



(5)

a) $R^1 = R^2 = H$
b) $R^1 = R^2 = Br$
c) $R^1 = Br, R^2 = H$
d) $R^1 = R^2 = Bu^t$
e) $R^1 = R^2 =$ 



(6)

a) R = H
b) R = Br
c) R = Bu^t
d) R =

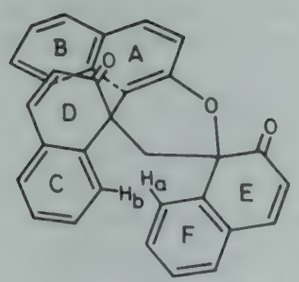


Figure 1—Most stable conformer of the compound (7a)

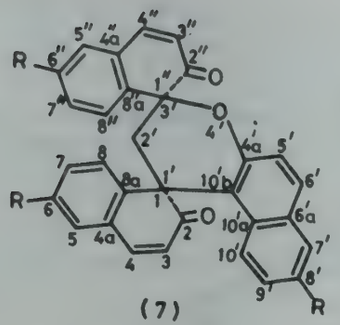
cis-configuration† was assigned to compound (C).

Hydrogenation of compound (**C**) resulted in a mixture of two products viz. ketone (**10a**) and the alcohol (**10b**). The preferential hydrogenation of one of the enone systems in compound (C) indicated that the other enone system is perhaps hindered on either side. Inspection of the Drieding models showed that in both conformers of the *cis*-isomer of compound (C), the E-ring enone system is free on either side while the D-ring enone is hindered (Fig. 1). But in the *trans* isomer, both the enone systems are almost equally hindered. Thus, the hydrogenation results are in support of the structure and configuration (**7a**) assigned to the compound (C).

The presence of two saturated carbonyls (IR: 1740 and 1725 cm^{-1}) and a group of multiplets (δ 4.05-4.18, 18 lines) integrating to four protons in PMR of compound (E) [*m/z* 440 (M^+)], clearly showed that this is the expected intramolecular cyclo addition product (**11a**). Thus, the photolysis of **1a** gave the novel compounds **(2a), (5a), (6a), (7a)** and **(11a)**.

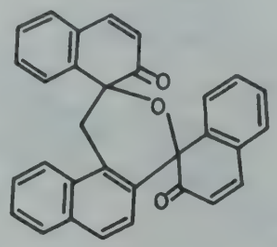
To study the generality of this reaction, the photolysis of substituted dispironaphthalenones (**1b-g**) was undertaken. Compound (**1b**) behaved exactly like **1a** in photolysis. On the other hand, while **1f** and **1g** on irradiation gave the corresponding dinaphthodioxins (**5**), quinone methide dimers (**6**) and cycloadducts (**11**), compounds (**1c-e**) gave only the corresponding dinaphthodioxins (**5**) and quinone methide dimers (**6**).

†In the earlier communication[7], a *trans*-configuration was assigned to this compound which has now been changed to *cis*.

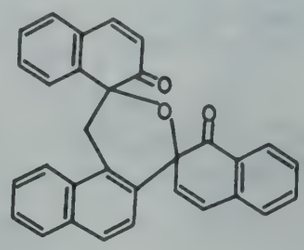


(7)

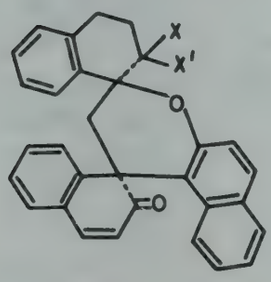
a) R = H ; b) R = Br



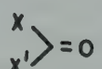
(8)

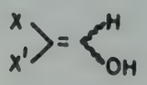


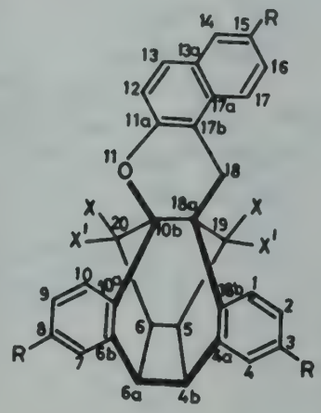
(9)



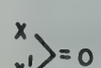
(10)

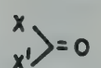
a) 

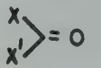
b) 

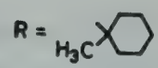
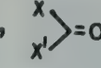


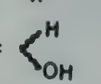
(11)

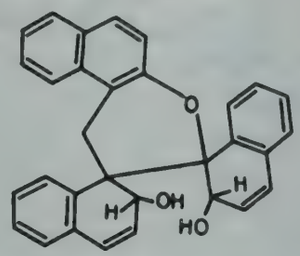
a) R = H , 

b) R = Br , 

c) R = Bu^t , 

d) R =  , 

e) R = H , 

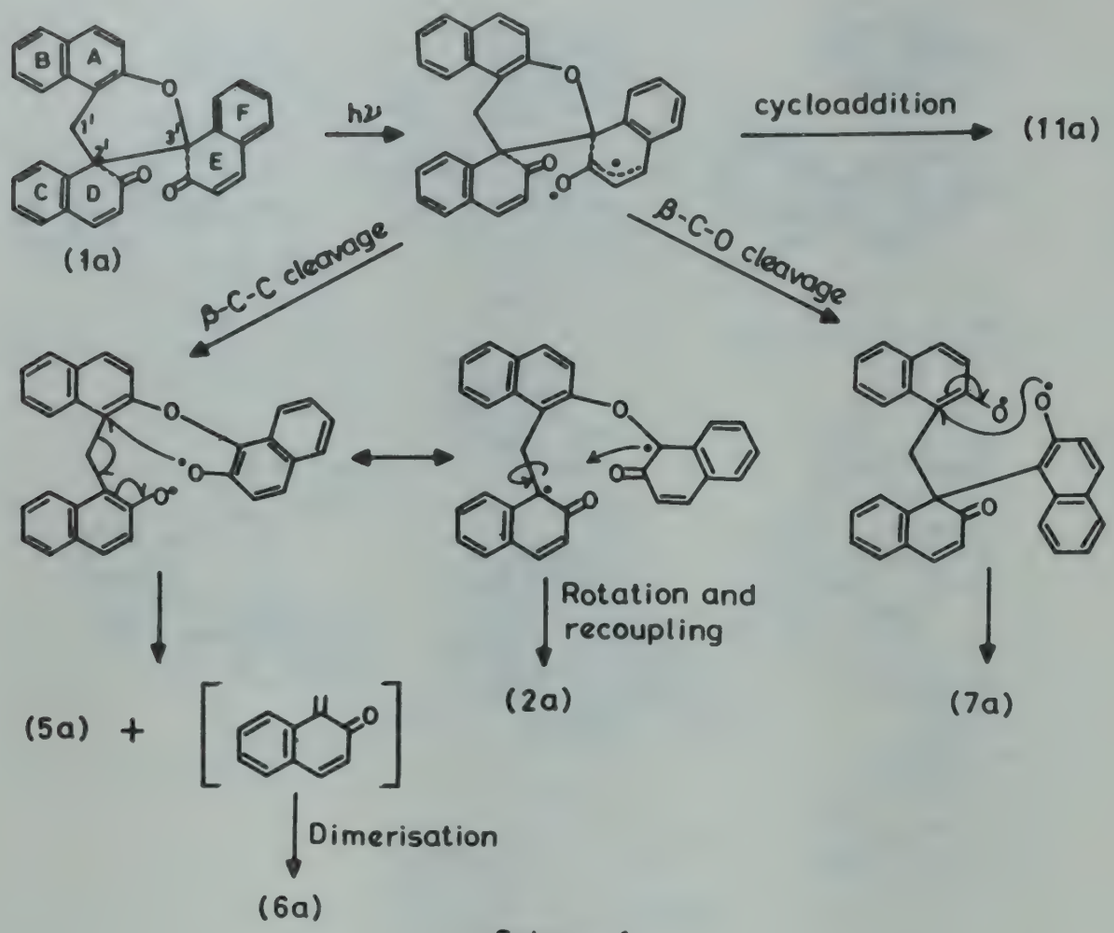


(12)

As already mentioned, β-cleavages in enones have been only rarely observed. In the case of **1a**, the β − C − C bond is highly stretched. Hence it may be expected that excitation of enone chromophore in **1a** might lead to the cleavage of β − C − C bond as the initial step. The tendency for this cleavage will be further enhanced by the stability of the resultant naphthoxy diradical. This diradical can recouple through the resonance α-keto carbon radical either directly to give back the starting **1a** or after rotation about C(1′)-C(2′) bond to give the trans-isomer (2a). As both **1a** and **2a** show almost similar absorptions above 300 nm and as **2a** was present in isolable amounts even after almost complete disappearance of the starting **1a**, the naphthoxy diradical is perhaps a triplet diradical which is unfavourable for immediate direct coupling. During the triplet lifetime available, the diradical perhaps undergoes rotation around C(1′)-C(2′) bond, followed by recoupling to give the *trans* isomer (**2a**)†. The naphthoxy diradical can form dinaphthodioxin (**5a**) by elimination of a quinone methide moiety which dimerises to **6a**. This appears to be the major reaction pathway for the naphthoxy diradical (Scheme 1).

Compound (**11a**) is formed obviously by intramolecular cycloaddition, probably by a radical pathway. Naphthalenone systems are known to undergo easy phtocycloaddition[8,9]. The much less efficiency of photocycloaddition in **1a** is perhaps due to the competing facile β-cleavage. Further, the intramolecular cycloaddition requires slight rotation around C(2′)-C(3′) bond to bring the enone π-bonds exactly parallel. This rotation might even facilitate the cleavage of the already weak C(2′)-C(3′) bond. If this is

†The photolysis of the *trans* dispironaphthalenone was carried out under similar conditions. Eventhough the presence of starting material was indicated (TLC), no isolable products were obtained which is difficult to explain.



Scheme 1

true, in the biallylic alcohol (**12**), photocycloaddition should be the sole process. Photolysis of the biallylic alcohol[10] (**12**) [UV($CHCl_3$): 337 (ε 6,216), 322 (1,776), 293 (7,104), 279 (12,432), 268 (14,208), 259 (15,984) and 243 nm (47,064)], gave mainly the cycloadduct (**11e**) with minor amounts of other products which were not investigated further.

The formation of **7a** can be visualised by an initial β−C−O cleavage on excitation of the enone chromophore to give a different naphthoxy diradical, which on recoupling through the naphthoxy radical of EF-ring and the α-keto carbon radical of AB-ring gives the compound (**7a**) (Scheme 1). It is interesting to note that the β−C−O bond in **1a**, which does not exhibit any unusual characteristics in the X-ray crystal structure analysis, also undergoes cleavage. This may be due to the stability of the resultant diradical.

Experimental Procedure

All melting points reported herein are uncorrected. UV and IR spectra were recorded on Shimadzu UV-190 and Perkin-Elmer 781 spectrophotometers respectively. PMR spectra were recorded either on a Jeol FX-90Q or a Bruker-WH-270 MHz FT NMR spectrometers and CMR spectra were recorded on a Bruker-WH-270 spectrometer at an operating frequency of 67.89 MHz. Chemical shifts are given as δ values (ppm) relative to TMS as internal standard. Unless otherwise stated, all irradiations were carried out using immersion type 450 W medium pressure mercury lamp with water cooled pyrex immersion-well, after bubbling nitrogen through the solution for 1-2 hr. Hexane refers to the fraction boiling in the range 60-80°.

Preparation of dispironaphthalenone (**1g**):
(i) *Bis*[6-(1-*methylcyclohexyl*)-2-*hydroxyl*-1-*naphthyl*]*methane* (**4b**)

A suspension of 6-(1-methylcyclohexyl)-2-naphthol[11] (24 g) in a solution of sodium sulphite (25.2 g) in water (150 ml) containing formaldehyde (40% aq; 7.0 ml) was heated (15 min, 100°) and the precipitate obtained was filtered off. Recrystallisation from benzene-hexane gave **4b** (12 g), m.p. 232-35°; IR (nujol): 3370 cm^{-1} (br, OH); PMR ($CDCl_3$, 90 MHz): 1.09 (6H, *s*, 2×Me), 1.18-2.22 (20H, *m*, 2×cyclohexyl), 4.66 (2H, *s*, CH_2), 7.09-7.73 (8H, *m*),, 8.18 (2H, *d*, *J*=8.8 Hz) and 9.92 (2H, *s*, 2×OH) (Found: C, 85.0; H, 8.2. $C_{35}H_{40}O_2$ requires C, 85.3; H, 8.2%).

(ii) *Oxidation of* **4b** *with DDQ*

A solution of **4b** (4.92 g) in dry benzene (400 ml) was refluxed with DDQ (2.5 g) for 10 hr, filtered, the filtrate concentrated and the residue chromatographed over neutral alumina (150 g). Elution with benzene followed by preparative TLC (silica gel, 2:1 benzene-hexane) gave the compound (**3b**) (550 mg), m.p. 154° (chloroform-hexane); IR (nujol): 1680 cm^{-1}; PMR ($CDCl_3$, 90 MHz): 1.19 (3H, *s*, Me), 1.25 (3H, *s*, Me), 1.32-2.35 (20H, *m*, 2 × cyclohexyl), 3.85 (2H, *ABq*, $\Delta\nu_{AB} = 48.7$ Hz, $J_{AB} = 18.0$ Hz), 6.37 (1H, *d*, $J = 10.0$ Hz) and 7.32-8.05 (9H, m) (Found: C, 85.3; H, 7.9. $C_{35}H_{38}O_2$ requires C, 85.7; H, 7.8%) and di(1-methylcyclohexyl)quinone methide dimer (**6d**) (500 mg), m.p. 180° (chloroform-hexane); IR (nujol): 1690 cm^{-1}; PMR ($CDCl_3$, 90 MHz): 1.20 (3H, *s*, Me), 1.25 (3H, *s*, Me), 1.35-3.24 (24H, *m*, 2 × cyclohexyl and 2 × CH_2), 6.11 (1H, *d*, $J = 9.9$ Hz) and 7.15-7.87 (9H, *m*) (Found: C, 85.4; H, 7.8. $C_{36}H_{40}O_2$ requires C, 85.7; H, 8.0%).

Further elution with chloroform gave *cis*-tri(1-methylcyclohexyl)dispironaphthalenone (**1g**) (700 mg), m.p. 182° (benzene-hexane); MS: *m/z* 728 (M^+); IR (nujol): 1680 and 1660 cm^{-1}; UV ($CHCl_3$): 338 (ε 9,360), 317 (14,560), 291 (10,660), 280 (13,520), 269 (16,250) and 244 nm (88,400), PMR ($CDCl_3$, 270 MHz): 1.02 (6H, *s*, 2 × Me), 1.22 (3H, *s*, Me), 1.27-2.10 (30H, *m*, 3 × cyclohexyl), 3.71 (2H, *ABq*, $\Delta\nu_{AB} = 272.7$ Hz, $J_{AB} = 17.9$ Hz), 5.74 (1H, *d*, $J = 10.0$ Hz), 5.93 (1H, *d*, $J = 9.6$ Hz), 6.17 (1H, *d*, $J = 8.3$ Hz), 6.61-6.66 (2H, *m*), 6.76 (1H, *d*, $J = 1.9$ Hz), 6.95-7.29 (5H, *m*), 7.50 (1H, *dd*, $J = 8.9$ and 1.9 Hz), 7.67 (1H, *d*, $J = 8.9$ Hz), 7.73 (1H, *d*, $J = 1.6$ Hz) and 7.76 (1H, *d*, $J = 9.0$ Hz) (Found: C, 85.4; H, 7.8. $C_{52}H_{56}O_3$ requires C, 85.7; H, 7.8%).

Irradiation of dispironaphthalenones (**1a-g**):

(i) *Dispironaphthalenone* (**1a**)

1a (450 mg) in dry benzene (100 ml) was irradiated for 7 hr during which time the dark yellow solution became pale. The solution was concentrated and chromatographed over neutral alumina. Elution with benzene-hexane (1:1), followed by removal of solvent gave the dinaphthodioxin (**5a**) (110 mg). Further elution with chloroform gave four compounds (B, C, D and E) were separated by repeated preparative TLC (silica gel, 4:1 chloroform-hexane) and further purified by crystallisation.

Compound (B) (55 mg) was identified as the known quinone methide dimer (**6a**). Compound (ε) (70 mg) [CMR ($CDCl_3$): 50.04 (*t*, C-2′), 55.00 (*S*, C-1 or C-1′), 82.46 (*S*, C-3′ or C-1″), 111.39 (*d*, C-5′), 111.98 (*S*, c-10′b), 123.17-144.50 (23 × C), 155.88 (*S*, C-4a′), 202.28 (*S*, C-2″) and 202.75 (*S*, C-2) (Found: C, 84.0; H, 4.7. $C_{31}H_{20}O_3$ requires C, 84.5; H, 4.6%)] was identified as *cis*-dispiro{naphthalene-1(2*H*)-1′-[1*H*]-naphtho[2,1-*b*]pyran-3′(2′-*H*), 1″(2″*H*)-naphthalene}-2(1*H*), 2″(1″*H*)-dione (**7a**).

Compound (D) (40 mg) was identified as the known *trans*-dispironaphthalenone (**2a**).

Compound (E) (35 mg) [(Found: C, 84.6; H, 4.7. $C_{31}H_{20}O_3$ requires C, 84.5; H, 4.6%] was identified as **4b**, 5, 6, 6a-tetrahydro-5, 18a: 6, 10b-dimethano-18*H*-dibenzo[3,4:7,8]cyclobuta[5,6]cycloocta[1,2-*b*]naphtho[1,2-*e*]pyran-19,20-dione (**11a**).

(ii) *Dispironaphthalenone* (**1b**)

(**1b**)[2] (350 mg) [UV($CHCl_3$): 360 (ε 4,760), 344 (7,620), 310 (14,760), 294 (13,800), 284 (14,760) and 247 nm (91,900)] in dry benzene (60 ml) was irradiated for 8 hr. The solution was concentrated and the white needles which separated were filtered and further purified by crystallisation from chloroform-hexane to give the dibromodinaphthodioxin (**5b**)[§] (85 mg), m.p. > 300°; MS: *m/z* 440 (M^+) (Found: C, 54.6; H, 2.8. $C_{20}H_{10}O_2Br_2$ requires C, 54.9; H, 2.5%). The mother liquor after separating **5b**, on preparative TLC (silica gel, benzene) gave *trans*-tribromo-dispironaphthalenone (**2b**) (35 mg), m.p. 175° (lit.[2] m.p. 175°); *cis*-tribromodispironaphthalenone (**7b**) (50 mg), yellow solid, m.p. 210° (chloroform-hexane); MS: *m/z* 674 (M^+); IR (nujol): 1695 and 1675 cm^{-1}; PMR ($CDCl_3$, 270 MHz): 2.67 (2H, *ABq*, $\Delta\nu_{AB} = 82.5$ Hz, $J_{AB} = 14.3$ Hz), 5.60 (1H, *d*, $J = 10.0$ Hz), 6.19 (1H, *d*, $J = 8.9$ Hz), 6.22 (1H, *d*, $J = 10.0$ Hz), 6.36 (1H, *d*, $J = 8.4$ Hz), 6.99 (1H, *d*, $J = 10.0$ Hz), 7.17 (1H, *dd*), 7.20 (1H, *dd*), 7.37 (1H, *d*, $J = 1.6$ Hz), 7.49 (1H, *d*, $J = 10.0$ Hz), 7.51 (1H, *d*, $J = 8.6$ Hz), 7.53 (1H, *dd*), 7.58 (1H, *d*, $J = 2.0$ Hz), 7.61 (1H, *d*, $J = 7.8$ Hz), 7.83 (1H, *d*, $J = 8.9$ Hz) and 7.97 (1H, *d*, $J = 1.6$ Hz) (Found: C, 54.9; H, 2.5. $C_{31}H_{17}O_3Br_3$ requires C, 55.0; H, 2.5%); dibromo quinone methide dimer (**6b**) (40 mg), m.p. 176° (lit.[12] m.p. 177°); and tribromo cycloadduct (**11b**) (25 mg), white needles, m.p. > 300° (chloroform-hexane); MS: *m/z* 674 (M^+); IR (nujol): 1745 and 1730 cm^{-1}; PMR ($CDCl_3$, 270 MHz): 3.53 (2H, *s*, CH_2), 4.00-4.19 (4H, *m*, cyclobutane protons), 6.54 (1H, *d*, $J = 8.1$ Hz), 7.01 (1H, *d*, $J = 8.4$ Hz), 7.10 (1H, *dd*), 7.31 (1H, *dd*), 7.33 (1H, *d*, $J = 1.6$ Hz), 7.37 (1H, *d*, $J = 2.0$ Hz), 7.43 (1H, *d*, $J = 8.9$ Hz), 7.68 (1H, *dd*), 7.72 (1H, *d*, $J = 8.9$ Hz), 7.91 (1H, *d*, $J = 8.9$ Hz) and 8.05 (1H, *d*, $J = 1.6$ Hz) (Found: C, 55.1; H, 2.7. $C_{31}H_{17}O_3Br_3$ requires C, 55.0; H, 2.5%).

[§]PMR spectrum could not be recorded due to the poor solubility in common organic solvents.

(iii) *Dispironaphthalenone* (**1c**)

1c[2] (200 mg) [UV($CHCl_3$): 360 (ε 5,000), 345 (8,000), 312 (10,000), 290 (14,000), 282 (13,500) and 246 nm (85,000)] in dry benzene (40 ml) was irradiated for 8 hr. The solution was concentrated and subjected to preparative TLC (silica gel, 1:1 benzene-hexane). Two compounds isolated were identified as the monobromo dinaphthodioxin (**5c**)[§] (60 mg), m.p. 295° (benzene-hexane); MS: *m/z* 362 (M^+) (Found: C, 66.2; H, 2.8. $C_{20}H_{11}O_2Br$ requires C, 66.4; H, 3.1%) and quinone methide dimer (**6a**) (35 mg).

(iv) *Dispironaphthalenone* (**1d**)

1d[2] (200 mg) [UV ($CHCl_3$): 360 (ε 3,850), 346 (8,200), 310 (11,600), 292 (14,500), 285 (13,500), 262 (21,500) and 244 nm (75,800)] in dry benzene (40 ml) was irradiated for 8 hr. The solution was concentrated and the residue, thus obtained, on subjecting to preparative TLC (silica gel, 1:1 benzene-hexane) gave the dibromo dinaphthodioxin (**5b**) (75 mg) and quinone methide dimer (**6a**) (25 mg).

(v) *Dispironaphthalenone* (**1e**)

1e[2] (200 mg) [UV)$CHCl_3$): 360 (ε 4,360), 346 (7,520), 310 (14,560), 292 (12,900), 283 (14,000) and 246 nm (81,900)] in dry benzene (40 ml) was irradiated for 8 hr and the solvent was completely removed. The residue, thus obtained, on preparative TLC (silica gel, 1:1 benzene-hexane) gave the monobromo dinaphthodioxin (**5c**) (60 mg) and dibromo quinone methide dimer (**6b**) (40 mg).

(vi) *Dispironaphthalenone* (**1f**)

1f[2] (450 g) [UV($CHCl_3$): 341 (ε 8,107), 318 (12,869), 290 (9,221), 278 (10,437) and 244 nm (75,291) in dry benzene (75 ml) was irradiated for 8 hr, the solvent was completely removed and the residue chromatographed over neutral alumina. Elution with benzene, followed by removal of solvent gave the di-*t*-butyldinaphthodioxin (**5d**) (120 mg), m.p. >300° (chloroform-hexane); MS: *m/z* 396 (M^+); PMR ($CDCl_3$, 270 MHz): 1.42 (18H, *s*, 2×Bu^t), 7.20 (2H, *d*, *J*=8.8 Hz), 7.45 (2H, *d*, *J*=8.8 Hz), 7.61 (2H, *dd*, *J*=8.9 and 1.9 Hz), 7.68 (2H, *d*, *J*=1.9 Hz) and 8.06 (2H, *d*, *J*=8.9 Hz) (Found: C, 84.5; H, 7.0. $C_{28}H_{28}O_2$ requires C, 84.8; H, 7.1%). Further elution with chloroform gave a mixture of two compounds which were separated by preparative TLC (silica gel, benzene) and further purified by crystallisation. They were identified as the di-*t*-butylquinone methide dimer (**6c**) (60 mg), m.p. 218° (d) [lit.[2] m.p. 218° (d)] and tri-*t*-butyl cycloadduct (**11c**) (90 mg), m.p. >300° (chloroform-hexane); MS: *m/z* 608 (M^+); IR ($CHCl_3$): 1720 and 1710 cm^{-1}; PMR ($CDCl_3$, 270 MHz): 1.18 (9H, *s*, Bu^t), 1.24 (9H, *s*, Bu^t), 1.46 (9H, *s*, Bu^t), 3.59 (2H, *s*), 3.98-4.18 (4H, *m*), 6.73 (1H, *d*, *J*=8.3 Hz), 6.96-7.16 (5H, *m*), 7.40 (1H, *d*, *J*=8.9 Hz), 7.67 (1H, *dd*, *J*=8.9 and 1.9 Hz), 7.76 (1H, *d*, *J*=8.9 Hz), 7.83 (1H, *d*, *J*=1.9 Hz) and 8.01 (1H, *d*, *J*=8.9 Hz) (Found: C, 84.7; H, 7.3. $C_{43}H_{44}O_3$ requires C, 84.9; H, 7.3%).

(vii) *Dispironaphthalenone* (**1g**)

1g (450 mg) in dry benzene (65 ml) was irradiated for 8 hr. The solution was concentrated and the white needles which separated were filtered and further purified by crystallisation from chloroform-hexane to give di-(1-methylcyclohexyl)dinaphthodioxin (**5e**) (95 mg), m.p. >300°; MS: *m/z* 476 (M^+); PMR ($CDCl_3$, 270 MHz): 1.26 (6H, *s*, 2×Me), 1.33-2.18 (20H, *m*, 2×cyclohexyl), 7.20 (2H, *d*, *J*=8.9 Hz), 7.45 (2H, *d*, *J*=8.9 Hz), 7.59 (2H, *dd*, *J*=8.9 and 1.5 Hz), 7.68 (2H, *d*) and 8.08 (2H, *d*, *J*=8.9 Hz) (Found: C, 85.4; H, 7.5. $C_{34}H_{36}O_2$ requires C, 85.7; H, 7.6%). The mother liquor after separating **5e**, on preparative TLC (silica gel, 3:1 benzene-hexane) gave di-(1-methylcyclohexyl)quinone methide dimer (**6d**) (45 mg) and tri-(1-methylcyclohexyl)cycloadduct (**11d**) (100 mg), m.p.>300° (chloroform-hexane); MS: *m/z* 728 (M^+); IR (nujol): 1740 and 1720 cm^{-1}; PMR ($CDCl_3$, 270 MHz): 1.05 (3H, *s*, Me), 1.09 (3H, *s*, Me), 1.18-2.23 (33H, *m*, 3×cyclohexyl and Me), 3.60 (2H, *s*), 3.98-4.17 (4H, *m*), 6.76 (1H, *d*, *J*=8.2 Hz), 6.97 (1H, *dd*, *J*=8.2 and 1.8 Hz), 7.03-7.16 (4H, *m*), 7.41 (1H, *d*, *J*=8.9 Hz), 7.65 (1H, *dd*, *J*=8.9 and 1.5 Hz), 7.76 (1H, *d*, *J*=8.9 Hz), 7.82 (1H, *d*, *J*=1.5 Hz) and 8.02 (1H, *d*, *J*=8.9 Hz) (Found: C, 85.5; H, 7.6. $C_{52}H_{56}O_3$ requires C, 85.7; H, 7.8%).

Hydrogenation of dispironaphthalenone (**7a**)

A solution of **7a** (100 mg) in dry ethyl acetate (50 ml) containing 10% Pd/C (30 mg) was stirred in H_2 atmosphere for 15 hr. The catalyst was filtered off, the solution concentrated and repeated preparative TLC (silica gel, chloroform) of the residue, yielded two compounds. The less polar compound (55 mg), m.p. 252° (Found: C, 84.5; H, 4.8. $C_{31}H_{20}O_3$ requires C, 84.1; H, 5.0%) was identified as 3″, 4″-dihydrospiro{naphthalene-1(2*H*), 1′-[1*H*][2,1-*b*]-pyran-3′(2′*H*), 1″(2″*H*)-naphthalene}-2,2″-dione (**10a**). The more polar compound, white solid (25 mg), m.p. 140-42° (Found: C, 84.3; H, 5.2. $C_{31}H_{24}O_3$ requires C, 83.8; H, 5.4%) was identified as 3″,4″-dihydro-2″-hydroxydispiro{naphthalene-1(2*H*), 1′-[1*H*]naphtho[2,1-*b*]pyran-3′(2′*H*),1″(2″*H*)-naphthalene}-2-one (**10b**).

Irradiation of biallylic alcohol (**12**)

12 (225 mg) in dry benzene (100 ml) was irradiated with Rayonet reactor fitted with RPR 3000 lamps for 5 hr. The solution was concentrated and the residue, subjected to preparative TLC (silica gel, 24:1 benzene-ethyl acetate). The major proudct isolated was identified as 4b,5,6,6a-tetrahydro-5,18a:6,10b-dimethano-18*H*-dibenzo[3,4:7,8]cyclobuta[5,6]cycloocta[1,2-*b*]naphtho[1,2-*e*]pyran-19-,20-diol (**11e**) (85 mg), m.p. 295-97° (chloroform-hexane); MS: *m/z* 444 (M^+); IR (nujol): 3310-3090 cm^{-1}; PMR ($CDCl_3$, 270 MHz): 3.45-3.76 (4H, *m*), 3.92-4.20 (5H, *m*, 4H + OH), 5.82 (1H, *d*, J = 12.0 Hz, OH), 6.50-6.86 (7H, *m*), 7.04 (1H, *dd*, J = 7.5 and 0.9 Hz), 7.41 (1H, *d*, J = 8.8 Hz), 7.50 (1H, *ddd*), 7.63 (1H, *ddd*), 7.85 (1H, *d*, J = 8.8 Hz), 7.95 (1H, *d*, J = 8.0 Hz) and 8.17 (1H, *d*, J = 8.5 Hz) (Found: C, 83.5; H, 5.5. $C_{31}H_{24}O_3$ requires C, 83.8; H, 5.4%).

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Chemistry of Ayurvedic Crude Drugs: Part VIII[a]—Shatavari-2: Structure Elucidation of Bioactive Shatavarin-I & other Glycosides[b,c]

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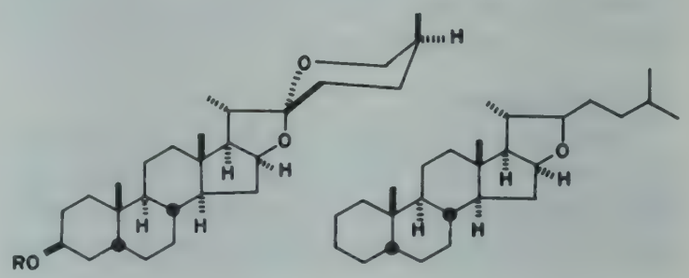
Shatavarin-I, the major glycosidal component of Ayurvedic crude drug *shatavari*, and possessing specific anti-oxytocin activity, is shown to be (25*S*,22*R*)-3-O-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside-26-O-β-D-glucopyranoside-5β-furostane-3β,22,26-triol (**4**).

Isolation of various glycosides of *shatavari* (*Asparagus racemosus*, Willd., decorticated roots)[1], a reputed Ayurvedic crude drug, and structure elucidation of one of these (shatavarin-IV) has been described earlier[2]. We now report on the structure of the biologically active component, shatavarin-I. Shatavarin-I in doses of 20-500 mcg/ml produces a specific and competitive block of oxytocin-induced contraction of rat, guinea pig and rabbit uteri *in vitro* as well as *in vivo*[3,4]. This compound also blocks spontaneous uterine motility in rats[5]. These activities have been interpreted in support of the Ayurvedic use of *shatavari* in cases of threatened abortion[2].



1: R = H

2: R = β-D-Glcp-(1→4)-β-D-Glcp-(1→)
α-L-Rhap-(1→2)

3

Shatavarin-I

Shatavarin-I could be obtained only as a foam (m.p. 184-87°; $[\alpha]_D$ −34.2°, MeOH). However its peracetylated product could be obtained crystalline (m.p. 119-21.5°). Saponification of crystalline peracetate led to shatavarin-I, again as a foam only. On acid hydrolysis (2*N* H_2SO_4, dioxane) shatavarin-I gave sarsasapogenin (**1**)[6] and sugars identified as glucose and rhamnose (paper chromatography). GLC of trimethylsilyl derivative of sugar mixture[1], carried out along with that of authentic sample, confirmed the nature of sugars and further revealed the presence of glucose and rhamnose in the ratio 3:1.

Unlike shatavarin-IV (**2**)[2], the IR spectrum of shatavarin-I does not exhibit the four-band absorption (1000-800 cm^{-1} region; 990, 920, 900, 850 cm^{-1}) considered to be characteristic of spiroketal side chain[7]. Thus, shatavarin-I is not a spirostane-based saponin and, in all probability, sarasasapogenin arises from a furostane (**3**) derivative during acid hydrolysis, and a number of such examples are known[8-10]. This conclusion is reinforced by the fact that shatavarin-I gives a red colour with Ehrlich's reagent, as expected of furostanols[11]. Elemental analyses of shatavarin-I and the derived peracetate are consistent with the molecular formula $C_{51}H_{86}O_{23}$ (furostanetriol + 3 glucose + 1 rhamnose $4H_2O$).

In an effort to confirm the above findings and extract additional structural information, mass spectra of shatavarin-I and its derivatives were investigated. Though, in the case of shatavarin-IV (**2**) electron-impact (EI) mass spectrometry had proved uniquely informative[1], shatavarin-I and its permethyl ether failed to volatalise even upto 350° and hence spectra could not be recorded. While considering alternative modes of mass spectrometry[12], the recently invented fast atom bombardment (FAB) mass spectrometry[13] which has been used with advantage for several classes of underivatized complex organic molecules, appeared most promising. In the first instance FAB MS of shata-

[a]Part VII: *Tetrahedron*, **43** (1987) (In press).
[b]Abstracted from the Ph D Thesis of Jayshree Joshi, M.S. University, Vadodara, 1984.
[c]MRC Communication No. 59.

Table 1—FAB MS Data for Shatavarin-IV and Shatavarin-I

Shatavarin-IV		Shatavarin-I	
Ion	*m/z* (%)	Ion	*m/z* (%)
$[M+H]^+$	887 (2.7%)	$[M+glycerol+H]^+$	1159 (0.55%)
$[M+Na]^+$	909 (11.4)%	$[M+Na]^+$	1089 (0.64%)
$[(M+H)$-Rha$]^+$	741 4.3%)	$[M+Na-H]^+$	1088 (0.99%)
		$[M+Na-2H]^+$	1087 (1.76%)
$[(M+H)$-Glc$]^+$	725 (7.7)%	$[(1087)$-Rha$]^+$	941 (0.4%)
$[(M+H)$-Glc-Rha$]^+$	579 (4.1%)	$[(1087)$-Glc-$H_2O]^+$	907 (0.25%)
		$[(1087)$-2Glc-Rha$]^+$	617 (0.28%)
$[(M+H)$-2Glc-Rha$]^+$	417 (67%)	$[(1087)$-2Glc-Rha-$H_2O]^+$	599 (0.34%)
		$[(1087)$-3Glc-Rha-$H_2O]^+$	437 (0.22%)
		$[M$-Glc-H_2O-$H]^+$	885 (0.24%)
		$[M$-3Glc-Rha-H_2O-$H]^+$	415 (1.74%)
		$[(M+H)$-2Glc-Rha-$H_2O]^+$	741 (0.22%)
		$[(M+H)$-2Glc-Rha-$H_2O]^+$	579 (0.49%)
		$[(M+H)$-3Glc-Rha-$H_2O]^+$	417 (0.82%)

Glc = 162 amu (cleavage with H transfer).
Rha = 146 amu (cleavage with H transfer)

varin-IV (**2**) was investigated††. The relevant data are presented in Table 1. It is clear from the relevant ions listed in Table 1 that FAB MS furnished not only the molecular weight (pseudomolecular ion, $M^+ + H$ at *m/z* 887) but also yielded ions arising by sequential loss of sugar moieties, consistent with its structure (**2**). FAB MS of shatavarin-I was next investigated. At the highest mass number, ion *m/z* 1159 is considered to be a pseudomolecular ion involving glycerol, which was used as the sample matrix. Likewise, ion *m/z*1087 is another pseudomolecular ion involving sodium ($M^+ + Na - 2H = 1087$). These ions indicate a molecular weight of 1066, which is consistent with the formula $C_{51}H_{86}O_{23}$. Ions at *m/z* 941 and 907 represent repsectively loss of rhamnose and glucose (also loss of H_2O) indicating that both rhamnose and glucose are terminal. From the ions listed in Table 1, it is clear that the loss of first glucose moiety is invariably accompanied by loss of one molecule of water. This is interpreted (considering the formation of sarsasapagenin on acid hydrolysis) to suggest one of the glucose moieties at C-22/C-26, leading to 5β-furostane-3β,22,26-triol as the aglycone of shatavarin-I. The group C set of ions (Table 1) may be considered as arising from sequential loss of sugar units after loss of first glucose (and water) from the substrate; these set of ions may be compared with ions at the same *m/z* arising from shatavarin-IV (Table 1). Keeping in mind the co-occurrence of shatavarin-I and shatavarin-IV, structure **4**, a possible progenitor of shatavarin-IV, appears to meet the structural requirements of shatavarin-I recognized so far. That this indeed represents, shatavarin-I has been established as follows.

Glycosides based on 5β-furostane-3,22,26-triol and β-glucosylated at C-26 have been selectively deglucosylated at C-26, either with a mild acid treatment or enzymatically with a β-glucosidase, to furnish spirostane derivatives with the glycoside linkage at C-3 intact[9,10,14]. When shatavarin-I is exposed to a β-glucosidase preparation, shatavarin-IV (**2**) and glucose are formed. This reaction not only establishes the sequence and nature of sugars at C-3, but also clarifies configuration at anomeric centres.

Compound-A

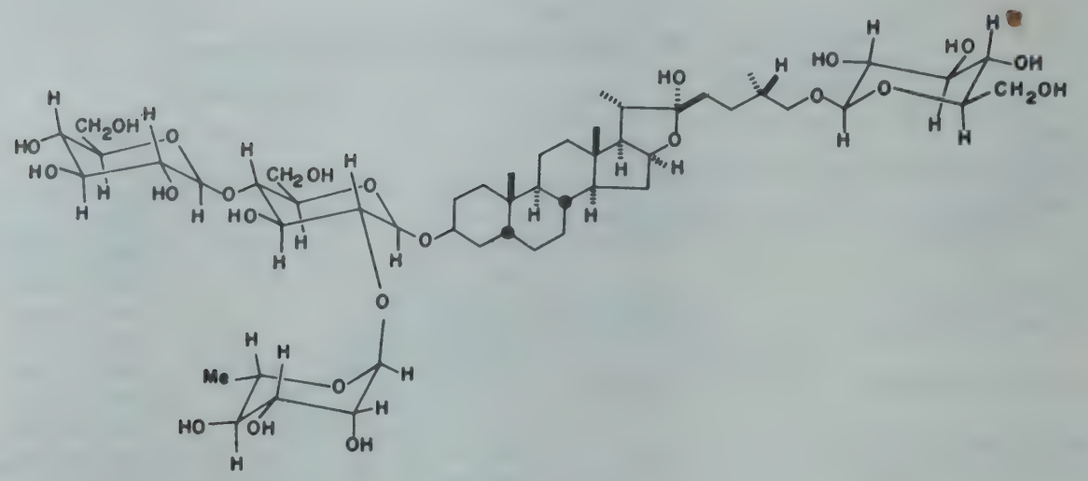
Compound-A, already described[2], was identified, based on its physical properties and spectral data, as *n*-butyl-β-D-fructopyranoside; compound-A is already known as a constituent of calyx of *Diospyros kaki*[16]. Compound-A is clearly an artefact as it could not be isolated when butanol was eliminiated from the extraction procedure[1].

Glycoside-AR-4

While evaluating a variety of *Asparagus racemosus* roots from different locations for their shatavarin-I and shatavarin-IV content, the material

†Though application of FAB mass spectrometry to the problem under discussion was obvious from published information available then (mid 1983), this technique had not been exploited for sequencing sugars in glycosides. This method had been mostly exploured for determination of molecular weight of complex organic molecules, except in the case of peptides[13e] where more extensive structure assigning capabilities had been exploited. Since then, publications dealing with sugar sequencing in glycosides have appeared[15].

‡FAB mass spectral data was provided by Dr R C Pandey. Full details will be published elsewhere.



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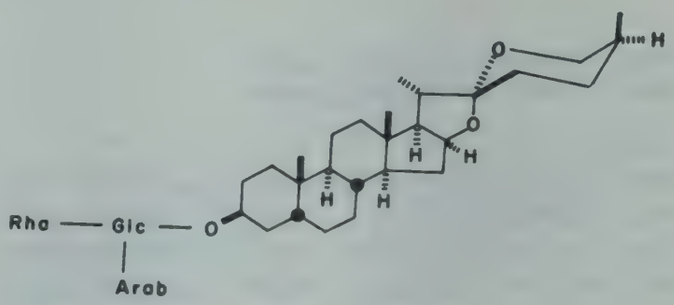
grown in the vicinity of Vadodara was also examined. Though this material displayed a spot (TLC) at the same R_f as that of shatavarin-IV, the compound on isolation proved to be different. This compound (glycoside-AR-4, m.p. 212-15°) on acid hydrolysis furnished sarsasapogenin (**1**), glucose, rhamnose and arabinose (paper chromatography; GLC of peracetates: ratio, 1:1:1). The IR spectrum of AR-4 displayed bands at 982, 917, 895 and 848 cm^{-1}, in which the intensity of 922 cm^{-1} band was stronger than that of 895 cm^{-1} band; these findings are characteristic of a spirostane[7] framework with *S* chirality at C-25. Thus AR-4 is based on sarsasapogenin with three different sugars attached in some sequence at C-3. Elemental analyses are consistent with the molecular formula $C_{44}H_{72}O_{16}$.

In the FAB MS of AR-4 the following ions are diagnostically important: *m/z* 857 (12.5%; pseudomolecular ion, $M^+ + 1$), 725 (1.5%; 857-Ara), 711 (2.1%; 857-Rha), 579 (2.3%; 857-Ara-Rha), 417 (13%; 857-Ara-Rha-Glc). These data lead to structure **5** for glycoside-AR-4. Because of paucity of material further work aimed at elucidating the points of linkage of arabinose and rhamnose to glucose could not be carried out.



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Experimental Procedure

All m.ps are uncorrected. Alumina for column chromatography (−80, +250 mesh) was treated with HNO_3 aq[17], washed neutral with water, dried, activated at 450° (5-6 hr), and standaridized[18]. Silica gel for column chromatography (−100, +200 mesh) was washed with hot water till sulphate-free, dried, activated at 125-30° (6 hr), and standardized[19]. TLC was carried out on SiO_2 gel layers (0.3 mm) containing 15% gypsum; visualization of spots was carried out by spraying with 1% vanillin in 50% H_3PO_4 aq, followed by heating (100°, 10 min). Paper chromatography (for sugars) was carried out with Whatman No. 1 paper by upward irrigation using organic phase of *n*-BuOH − AcOH − H_2O (4:1:5), visualization was done by spraying with aniline hydrogen phthalate[20].

Following instruments were used for spectral/analytical data: Schmidt + Haensch electronic polarimeter, model Polartronic I; Perkin-Elmer infrared spectrophotometer, model 577; Perkin-Elmer NMR spectrometer, model R32 (90 MHz) (^{1}H NMR); Jeol FX-100 (^{13}C NMR); Hewlett-Packard gas chromatograph, model 700 or 5712 A. FAB MS were recorded on VG Micromass 7070E-HF mass spectrometer; Xenon source (8 KeV) was used to bombard the sample dissolved in glycerol.

Shatavarin-I

Amorphous powder, m.p. 184-87°, $[\alpha]_D$ − 34.2° (MeOH, *c* 1.0%). IR (KBr): 3400, 1620 (bound H_2O), 1165-985 cm^{-1} (unresolved). (Found: C, 55.6; H, 8.2. $C_{51}H_{86}O_{23}$ $2H_2O$ requires C, 55.5; H, 8.2%).

Tridecaacetylshatavarin-I

Shatavarin-I (152 mg) in dry pyridine (10 ml) was reacted with acetic anhydride (10 ml) at room temperature (26-31°) for 48 hr and worked up in the usual manner to get a product (200 mg; m.p.

114-17°, $[\alpha]_D$ −19.8° in $CHCl_3$) which was repeatedly crystallized from dry ethanol to get a crystalline solid, m.p. 119.5-21°; IR(KBr): 3440, 1745, 1220, 1035, 900 cm^{-1}; ^1H NMR($CDCl_3$): *OAc* (singlets at 1.95, 1.99, 2.02, 2.03, 2.04, 2.06, 2.08, 2.09, 2.14, 2.16 ppm, total 39H), C*H*OR (complex *m*, 3.50-5.20 ppm, 28H) (Found: C, 57.7; H, 7.4. $C_{77}H_{112}O_{36}$ requires C, 57.3; H, 7.0%).

Tetradeca-O-methylshatavarin-I

Shatavarin-I (1.5 g) was dissolved, at room temp (~28°), in DMSO (40 ml; dried by distillation over CaH_2), and to the stirred solution NaH (50% dispersion in paraffin, 1.5 g; washed with pet ether thrice before use) was added, followed after 1/2 hr with MeI (10 ml), under cooling (~20°). After stirring for another 1 hr at room temp, water (200 ml) was added and the product taken up in ether (50 ml×6). The combined ether extract was washed with water (50 ml×6), dried (Na_2SO_4) and freed of solvent to get a product (1.6 g), which was treated with NaH and MeI, as before, thrice. The final product (1.5 g) was purified by chromatography over SiO_2-gel/IIA (1.5 cm×60 cm) using $CHCl_3$ and 1% acetone in $CHCl_3$ as successive eluates. The TLC pure material was obtained as a foam, m.p. 68-70°, $[\alpha]_D$ −33.5° ($CHCl_3$, *c* 1.0%).

IR(KBr): no OH absorption, 975-1275 cm^{-1} unresolved absorption. (Found: C, 62.3; H, 8.8. $C_{65}H_{114}O_{23}$ requires C, 62.0; H, 9.1%).

Hydrolysis of shatavarin-I

Shatavarin-I (350 mg) in 2*N* H_2SO_4 (30 ml) and dioxane (30 ml) was refluxed (N_2) for 4 hr. The reaction mixture was cooled, diluted with water (150 ml) and extracted with benzene (100 ml×4). The combined benzene extract was washed with water (100 ml×4), brine and dried (Na_2SO_4). Solvent removal furnished a product (120 mg), which was purified by chromatography (SiO_2 gel/IIA, 0.9 cm×18 cm). 3% EtOAc in benzene (15 ml×6) eluted the major product (90 mg), which was repeatedly recrystallized from MeOH to get pure genin (55 mg, m.p. 197-200°, $[\alpha]_D$ −75° in $CHCl_3$) identified (m.m.p., IR, PMR) as sarsasapogenin (**1**) by comparison with an authentic sample.

The aq phase from the above work-up was neutralized by passing through a bed of anion exchange resin (IR-400, OH form; 300 ml), and then freed of water under suction to get the sugars (140 mg). Paper chromatography showed two spots identified by reference to authentic samples as glucose and rhamnose. Another portion (10 mg) of the total sugars was treated with dry pyridine (0.5 ml), trimethylsilyl chloride (0.2 ml) and hexamethyldisilazane (0.4 ml). The reaction mixture was shaken for 5 min and then freed of pyridine etc. (~60° under reduced pressure) and the residue in pet ether analyzed by GLC[21] (6ft column, 10% SE-30 on Chromosorb-W, 190°, 80 ml H_2/min). For comparison purposes, authentic D-glucose and L-rhamnose in proportion of 3:1 were silylated and analyzed by GLC.

Enzymic hydrolysis of shatavarin-I

Shatavarin-I (30 mg) in water (0.5 ml) was mixed with 0.05 *M* acetate buffer (*p*H 5.0; 0.5 ml) and a crude β-glucosidase preparation (*ex Fusarium moniliforme* Sheldon; 1 ml). The mixture was incubated at 50° for 24 hr, after which it was diluted with water (20 ml) and extracted with *n*-butanol saturated with water (25 ml×3). Solvent was removed from the combined solvent extract to get a product (m.p. 266-71°) which was identified as shatavarin-IV (**2**) by mixed m.p. (m.m.p. 266-73°; m.p. of shatavarin-IV, 274-78°. Highly purified shatavarin-IV has m.p. 307-9°) and TLC.

The aq part was shown to contain only glucose by paper chromatography.

Compound-A (n-Butyl-β-D-fructopyranoside)

Melting point 152-54.5°, $[\alpha]_D$ −140° (MeOH, *c* 0.9%); IR(KBr): 3350, 1365, 1330, 1250, 1185, 1170, 1145, 1115, 1055, 1010, 950, 900, 855, 765 cm^{-1}; ^1H NMR (D_2O): CH_3CH$_2$ (3H, 0.76 ppm), CH_2O (2H, *t*, 3.70 ppm; 4H, essentially *s*, 3.97 ppm), C*H*O (3H, essentially singlets at 4.10 and 4.16 ppm); ^{13}C NMR (D_2O): 13.88, 19.47, 32.07, 61.53, 62.12, 64.53, 69.14, 69.89, 70.41, 101.17 ppm; MS: *m/z* 205 (76%), 163 (22%), 149 (100%), 145 (20%), 103 (75%), 85 (52%), 77 (75%), 73 (68%), 69 (22%), 60 (73%), 57 (59%) (Found: C, 50.7; H, 8.6. $C_{10}H_{20}O_6$ requires C, 50.8; H, 8.5%).

Isolation of glycoside-AR-4

Fresh roots (60 kg) of *Asparagus racemosus* grown in Vadodara were harvested in November-December and dried in shade, after being cut into small pieces (time taken for drying 15 days, weight loss 91%). This material was powdered and 2 kg of it were extracted twice with hot acetone (6.5 1×2) in a Soxhlet-type apparatus for 48 hr each time to get, after solvent removal 38 g of a dark brown gum. The marc was next similarly extracted with hot 95% EtOH to furnish 345 g of extract as a dark brown hygroscopic foam. This product (315 g) was dissolved in water (420 ml) and the soln extracted with *n*-butanol saturated with water (220 ml×8). The combined butanol extract was

freed of solvent to get the crude saponins as a dark brown foam (69 g).

This material (30 g) was chromatographed on Al_2O_3/IV (1 kg, 4.5 cm × 70 cm) using *n*-BuOH satd with water as the eluant, while monitoring with TLC (solvent: $CHCl_3 - MeOH - H_2O$, 65:35:10, lower phase). Fraction (3.9 g) having the same R_f as that of shatavarin-IV[2] was rechromatographed over SiO_2-gel/IIA (100 g, 2.0 cm × 56 cm) using $CHCl_3$, followed by $CHCl_3$ containing increasing amounts of MeOH (4 to 40%) as eluants. 30% MeOH in $CHCl_3$ (100 ml × 10) eluted a product (1.17 g), which was repeatedly crystallized from hot MeOH to get glycoside-AR-4 as a white crystalline powder (300 mg), m.p. 212-15° (same R_f as that of shatavarin-IV); IR(KBr): 3400, 1630, 1130, 1090, 1060, 1045, 982, 913, 895, 848, 805, 778 cm^{-1} (Found: C, 59.8; H, 8.6. $C_{45}H_{74}O_{16} \cdot H_2O$ requires C, 60.4; H, 8.5%).

Hydrolysis of glycoside-AR-4

Glycoside-AR-4 (20 mg) was heated (4 hr) on a steam-bath with 2*N* H_2SO_4 (7 ml) and dioxane (3 ml). Work-up as already described for shatavarin-I led to genin (4 mg), identified as sarasasapogenin (**1**), and sugars (10 mg), shown by paper chromatography as glucose (R_f 0.20), arabinose (R_f 0.25) and rhamnose (R_f 0.36), by reference to authentic samples.

The sugar mixture (10 mg) was acetylated (dry pyridine, 0.5 ml; Ac_2O, 0.5 ml) at 145-50° (4 hr). The product on GLC (10% DCQF-1 on 60-80 mesh Chromosorb-W, temp 210°) displayed three peaks (major anomers) at RRT of 1, 1.3 and 3.27 in the ratio 1:1:1. These were identified respectively as rhamnose, arabinose and glucose by reference to authentic samples.

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Configurations at C-24 of 24-Alkylsterols from *Clerodendrum infortunatum* Linn.

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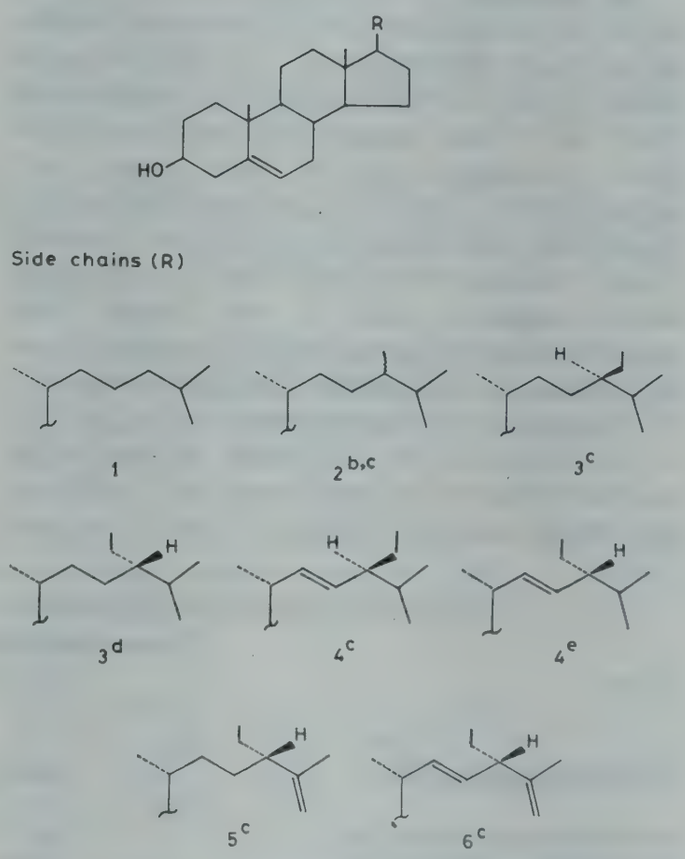
The configurations at C-24 of five 24-alkyl-Δ^5-sterols isolated from the aerial parts of *Clerodendrum infortunatum* have been determined with the help of high-resolution ^{1}H NMR spectroscopy. Two major 24-ethylsterols which possess a Δ^{25}-bond (24-ethyl-25-dehydrocholesterol and 24-ethyl-22,25-bisdehydrocholesterol) have been determined to be the 24β-epimers, while the two minor 24-ethylsterols which lack a Δ^{25}-bond (24-ethylcholesterol and 24-ethyl-22-dehydrocholesterol) have been shown to be the 24α-epimers. On the other hand, 24-methylcholesterol, another minor sterol, has been found to be the C-24 epimeric mixture containing predominantly the 24α-epimer.

Clerodendrum infortunatum Linn. (Verbenaceae) contains clerosterol, the structure of which was shown to be 24-ethylcholesta-5,25-dien-3β-ol(24-ethyl-25-dehydrocholesterol) (**5**), a 24-ethylsterol possessing a Δ^{25}-bond, without proof for the configuration at its C-24[1]. The only other sterol detected so far in this plant is the 22-dehydro derivative[§] of **5**, which possesses the C-24β-configuration and is formulated as 24β-ethylcholesta-5,22,25-trien-3β-ol(24β-ethyl-22,25-bisdehydrocholesterol)[24β-(**6**)][2]. Since majority of higher plants contain 24-alkylsterols, which lack a Δ^{25}-bond, as the major sterols represented by sitosterol (24α-ethylcholest-5-en-3β-ol) [24α-(**3**)][3,4], the presence of 24-ethyl-Δ^{25}-sterols predominantly in *C. infortunatum* and in some other *Clerodendron* species[2,5] constitutes the very characteristic feature of this species. Thus, we considered it worthwhile to carry out a detailed analysis of the sterol constituents of *Clerodendrum* species. This paper describes a thorough investigation on the sterols of the leaves and stems of *C. infortunatum* Linn. which led to the isolation and identification of four sterols in addition to the above mentioned two 24-ethyl-Δ^{25}-sterols (**5** and **6**). The configurations at C-24 were determined for five 24-alkylsterols with the help of 250 MHz ^{1}H NMR spectroscopy.

The air-dried and powdered (5 kg) aerial parts (leaves and stems) of *C. infortunatum*, which was collected locally in India, were extracted with $CHCl_3$ in a soxhlet extractor for 40 hr. Saponification of the crude extract (232 g) with 5% ethanolic KOH for 24 hr at room temperature followed by extraction with diethyl ether gave the unsaponifiable lipid (64.5 g). The unsaponifiable lipid was subjected to column chromatography on silica gel (*n*-hexane-Et_2O, *n*-hexane-EtOAc, and the MeOH as eluant, monitoring by TLC) to give the sterol fraction (2.6 g, R_f = 0.19 on TLC). Acetylation of the sterol fraction gave an acetate fraction (2.6 g) which was subjected to argentation



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§All sterol possessing a Δ^{22}-bond described here have a *trans*-configuration at C-22.

TLC to give four separated bands (refereed to as bands 1-4 in he order of polarity, beginning with the least polar). The fraction (30 mg) from band-1 was a mixture of three components which was subjected to further fractionation by HPLC giving three compounds: the acetates of **1** (1 mg), **2** (3 mg) and **3** (7 mg). Bands 2,3 and 4 provided the acetates of **4** (29 mg), **5** (216 mg) and **6** (604 mg), respectively. The melting points and the chromatographic and mass spectral data of these sterols, as have been shown in the experimental section, were consistent with those of the corresponding authentic compounds, and hence the sterols **1- 6** were identified. The relative abundance of each sterol in the sterol mixture of *C. infortunatum* was determined as: **1** (0.4%), **2** (0.8%), **3** (7.0%), **4** (3.3%), **5** (31.3%) and **6** (56.9%), based on GLC and argentation TLC data.

The confirmation of structures and the determination of configurations at C-24 of the 24-alkylsterols were performed by ^{1}H NMR analysis. Table 1 bears ^{1}H NMR data of the 24-alkylsterols (**2-6**) which were assigned by comparison with those of literature data[6-10]. The signals for **2** acetate from *C. infortunatum* were identical with those of its corresponding 24α-epimer. However, it also exhibited weak signals due to 24β-epimer which suggested that **2** was mostly 24α-methylcholesterol (campesterol) containing a minor amount of 24β-epimer. The ratio between the C-24 epimers of **2** was estimated to be 24α: 24β = 8:2 based on the peak height of the 21-CH_3 doublet[10]. The two 24-ethylsterols of *C. infortunatum* which lack a Δ^{25}-bond (**3** and **4** as acetyl derivatives) showed the ^{1}H NMR signals almost identical with those of the corresponding 24α-epimers but differed from those of the 24β-epimers especially in the resonance position of the 29-CH_3 triplet, and hence, these were identified as the acetates of sitosterol [24α-(**3**)] and stigmasterol [24α-(**4**)] respectively. On the other hand, the two 24-ethylsterols which possess a Δ^{25}-bond (**5** and **6** as acetyl derivatives) showed the ^{1}H NMR signals consistent with the corresponding 24β-epimers. However, the configurations at C-24 of **5** and **6** remained undetermined because of the non-availability of ^{1}H NMR data of the 24α-epimer of the sterols with a 24-ethyl-Δ^{25}-side chain, and also because both of the C-24 epimers of sterols possessing a 24-ethyl-$\Delta^{22,25}$ side chain exhibit very similar ^{1}H NMR signals[8]. Thus, the Δ^{25}-unsaturated sterols (**5** and **6**) were hydrogenated to convert them into C-25 saturated sterols. Hydrogenation of **5** acetate yielded the dihydro derivatives ($M^+ - HOAc$, *m/z* 396) quantitatively, the ^{1}H NMR data (Table 1) of which were very much similar to those of clionasterol [24β-(**3**)] acetate but differed from those of the 24α-epimer. Hence, **5** was regarded as the 24β-epimer, 24β-ethyl-25-dehydrocholesterol [clerosterol, 24β-(**5**)]. Hydrogenation of **6** acetate afforded the dihydro ($M^+ - HOAc$, *m/z* 394) and tetrahydro ($M^+ - HOAc$, *m/z* 396) derivatives in the ratio of 4:6. The ^{1}H NMR data (Table 1) of the dihydro and tetrahydro derivatives were consistent with the 24β-epimers, the acetates of poriferasterol [24β-(**4**)] and clionasterol [24β-(**3**)], respectively, and hence, **6** isolated from *C. infortun-*

Table 1 — ^{1}H NMR Spectral Data of the Acetyl Derivatives of Some 24-Alkylsterols

[Chemical shifts in δ, ppm; *J* values in Hz][a]

Acetate		18-CH_3 (*S*)	19-CH_3 (*S*)	21-CH_3 (*d*,*J*=6.4)	26-CH_3 (*d*,*J*=6.7)	27-CH_3 (*d*,*J*=6.7)	28-CH_3 (*d*,*J*=6.0)	29-CH_3 (*t*,*J*=7.3)
	24α			0.910	0.851	0.801	0.773	
24-Methylcholesterol (**2**)[b,c]		0.676	1.017					—
	24β			0.918	0.856	0.781	0.775	
24α-Ethylcholesterol[24α-(**3**)][c]		0.677	1.017	0.920	0.836	0.813	—	0.844
24β-Ethylcholesterol[24β-(**3**)][d]		0.677	1.019	0.926	0.831	0.812	—	0.854
24α-Ethyl-22-dehydrochlesterol [24α-(**4**)][e]		0.696	1.021	1.022	0.844	0.795	—	0.805
24β-Ethyl-22-dehydrocholesterol [24β-(**4**)][e]		0.695	1.021	1.024	0.844	0.792	—	0.811
24β-Ethyl-25-dehydrocholesterol [24β-(**5**)][c]		0.668	1.015	0.904	1.562(*s*)	4.639(*s*,1H)	—	0.800
						4.724(*s*,1H)		
24β-Ethyl-22,25-bisdehydrocholesterol [24β-(**6**)][c]		0.690	1.019	1.008	1.646(*s*)	4.696(*s*,2H)	—	0.831

[a]Following signals were also observed: 2.028 - 2.032 (3H, *s*, 3β-OCOMe), 4.57-4.61 (1H,*m*, 3α-H), and 5.36 - 5.37 (1H, *m*,6H) for all compounds; 5.09 (2H, *m*, 22-H and 23-H) for the acetates of 24α- and 24β-(**4**); 5.18 (2H, *m*, 22-H and 23-H) for 24β-(**6**) acetate.

[b]C-24 Epimeric mixture (24α:24β = 8:2).

[c]Isolated from *C. infortunatum* in this study.

[d]Derived from 24β-(**5**) acetate by hydrogenation.

[e]Derived from 24β-(**6**) acetate by hydrogenation.

atum was considered to be the 24β-epimer, 24β-ethyl-22,25-bisdehydrocholesterol [22-dehydroclerosterol, 24β-(**6**)].

This study has thus demonstrated the presence of five sterols which lack a Δ^{25}-bond, i.e. **1**, 24α- and 24β-(**2**), 24α-(**3**), and 24α-(**4**), as the minor sterol constituents, besides the major 24-ethylsterols possessing a Δ^{25}-bond, 24β-(**5**) and 24β-(**6**), in *C. infortunatum*. The stereochemical features at C-24 of the minor 24-alkylsterols (**2-4**) were consistent with the sterols which are known to occur in majority of the higher plants[3,4]. Though sterol **5** was isolated from *C. infortunatum* for the first time, the C-24 configuration remained undetermined[1]. The co-existence of 24α- and 24β-ethylsterols in a higher plant has previously been demonstrated in the family Cucurbitaceae[6,8,11,12], and this study seems to be the second report on the detection of both 24α- and 24β-ethylsterols in a higher plant (*C. infortunatum*). From the viewpoint of sterol biogenesis, it may suggest the occurrence of the 24α-ethyl pathway leading to 24α-(**4**) through 24α-(**3**), which is considered to be the common pathway in most of the higher plants[3], in addition to the major 24β-ethyl pathway leading to 24β-(**6**) through 24β-(**5**), and the 24α- and 24β-methyl pathways in the tissues of *C. infortunatum*[3,7]. The presence of 24β-(**5**) and 24β-(**6**) is also known in *Kalanchoe daigremontiana* (Crassulaceae)[5,9], and it is highly probable that some 24α-ethylsterols may also occur in *K. daigremontiana*.

Experimental Procedure

Crystallizations were performed in acetone-methanol. Melting points were taken on a heated block and are uncorrected. Thin layer chromatography on silica gel was performed by developing the chromatoplates three times using *n*-hexane-ethyl acetate (6:1) as irrigant. Argentation ($AgNO_3$-silica gel, 1:4) TLC plates were developed four times with CCl_4-CH_2Cl_2 (5:1). High-performance liquid chromatography (HPLC) was carried out on a Partisil 5 ODS-2 column (25 cm × 10 mm i.d.) with MeOH as a mobile phase (flow rate, 4 ml/min) using an RI detector. GLC was performed on a SCOT OV-17 glass capillary column (30 m × 0.3 mm i.d., column temp. 260°). R_c values on argentation TLC and relative retention time (RRT) values on HPLC and GLC were taken relative to cholest-5-en-3β-ol(cholesterol) (**1**) acetate. Mass spectra (EI-MS, 70 ev) were taken by means of a probe injection. ^1H NMR spectra (250 MH_z) were recorded in $CDCl_3$ using TMS as internal standard. Acetylation was carried out with acetic anhydride-pyridine overnight at room temperature. Hydrogenation was performed overnight in ethanol over pre-reduced PtO_2 at ambient pressure and temperature. Authentic samples of **1**, the C-24 epimeric mixture of 24-methylcholest-5-en-3β-ol (24-methylcholesterol) (**2**), 24α-(**3**) and its 24β-epimer (clionasterol), 24α-(stigmasterol) and 24β-(poriferasterol) epimers of 24-ethylcholesta-5,22-dien-3β-ol(24-ethyl-22-dehydrocholesterol) (**4**), 24β-(**5**) and 24β-(**6**) were used in this study[6,13].

Physical, chromatographic and mass spectral data of C. infortunatum sterols

The melting points and chromatographic and mass spectral data of the acetyl derivatives of the sterols (**1-6**) isolated from *C. infortunatum* are given below:

(**1**)- Acetate: m.p. 113-16°; RRT = 1.00 (GLC), 1.00 (HPLC); R_c = 1.00; MS = *m/z* 368 (M^+ − HOAc; 100%), 353(29), 260(23), 255(28), 247(33), 228(6), 213(27).

(**2**) − Acetate: m.p.139-40°; RRT = 1.31 (GLC), 1.07 (HPLC); R_c = 1.00; MS = *m/z* 442 (M^+; 1%), 382 (100), 367(12), 274(8), 261(8), 255(12), 213(9).

(**3**)-Acetate: m.p.113-16°; RRT = 1.63 (GLC), 1.18 (HPLC); R_c = 1.00; MS = *m/z* 456 (M^+; 3%), 396(100), 381(11), 288(8), 275(8), 255(13), 227(3), 213(10).

(**4**)-Acetate: m.p. 145-46°; RRT = 1.43 (GLC); R_c = 0.96; MS = *m/z* 394 (M^+ − HOAc, 100%), 379(3), 351(11), 282(6), 255(31), 228(8), 213(8).

(**5**) − Acetate: m.p. 135-37°; RRT = 1.63 (GLC); R_c = 0.36; MS = *m/z* 394 (M^+ − HOAc, 100%), 379(12), 310(8), 286(5), 281(7), 273(8), 255(12), 228(12), 213(10).

(**6**) − Acetate: m.p.115-16°; RRT = 1.49 (GLC); R_c = 0.14; MS = *m/z* (M^+, 0.3%), 409(0.2), 392(100), 377(3), 363(3), 282(5), 255(32), 253(12), 228(3), 213(7).

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Studies on Rutaceae: Part VI†—Reactions & Rearrangements of Coumarins

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Interesting reactions and rearrangements of isoprenoid systems have been observed in naturally occurring compounds like 6-(3′,4′-epoxy-3′-methyl-γ-butyrolactonyl)-7-methoxy coumarin (micromelumin) (**1**), 7-methoxy-8-(1′,2′-epoxy-3′-methyl but-3′-enyl)coumarin (phebalosin) (**2**), 7-methoxy-8-(2′-hydroxy-3′-methyl but-3′-enyl)coumarin (auraptenol) (**3**) and 7-methoxy-8-(3′-methyl but-2′-enyl)coumarin (osthol) (**4**). These reactions/rearrangements have led to the synthesis of coumarins already isolated from nature. Micromelumin (**1**) with pyridinium chloride affords the naturally occurring angelical (**5**), 6-(3′-methyl-3′-chloro-4′-hydroxy-γ-butyrolactonyl)-7-methoxy coumarin (**6b**), 7-methoxycoumarinyl-6-tetronic acid derivative (**7**) and 6-(1′-carboxy-2′-methyl-3′,4′-dihydroxybutenyl)-7-methoxycoumarin (**8**). Phebalosin (**2**) has been used for the hemisynthesis of the two naturally occurring diastereoisomeric coumarins minumicrolin (**9**) and murrangatin (**10**). Osthol (**4**) and auraptenol (**3**) have been used for the synthesis of the naturally occurring arnottinin (**14**).

The coumarins are ubiquitous and are widely distributed in the plant kingdom, specially in the families Umbelliferae and Rutaceae. These constitute a fascinating class of natural products which exhibit varied number of biogenetic modifications of the simple isoprenoid unit either as C-6 and/or C-8 substituents. These substituents are envisaged to undergo interesting reactions/rearrangements leading to either naturally occurring coumarins or to new reactions. In the course of our studies on the chemistry of isoprenoid systems four naturally occurring coumarins were selected viz 6-(3′,4′-epoxy-3′-methyl-γ-butyrolactonyl)-7-methoxy coumarin (micromelumin) (**1**), a major constituent of *Micromelum pubescens* Blume, 7-methoxy-8-(1′,2′-epoxy-3′-methyl but-3′-enyl)coumarin (phebalosin) (**2**) and 7-methoxy-8-(2′-hydroxy-3′-methyl but-3′-enyl)coumarin (auraptenol) (**3**), occurring abundantly in *Murraya exotica* Linn. and 7-methoxy-8-(3′-methyl but-2′-enyl)coumarin (osthol) (**4**), isolated from *Feronia limonia* Linn.

Micromelumin (**1**) containing an epoxy-lactone system at C-6 was found to be vulnerable and afforded products arising not only by the opening up of the epoxide ring, but also by the degradation of the lactone system[1-4]. With pyridinium chloride **1** afforded four products **5, 6b, 7** and **8** (Chart 1). Compound **5** was (m.p., m.m.p., CO-IR, CO-PMR) with an authentic sample of naturally occurring angelical[5,6]. The structure of **6b** was settled from spectral studies and also from its transformation reaction. Its 400 MHz PMR spectrum

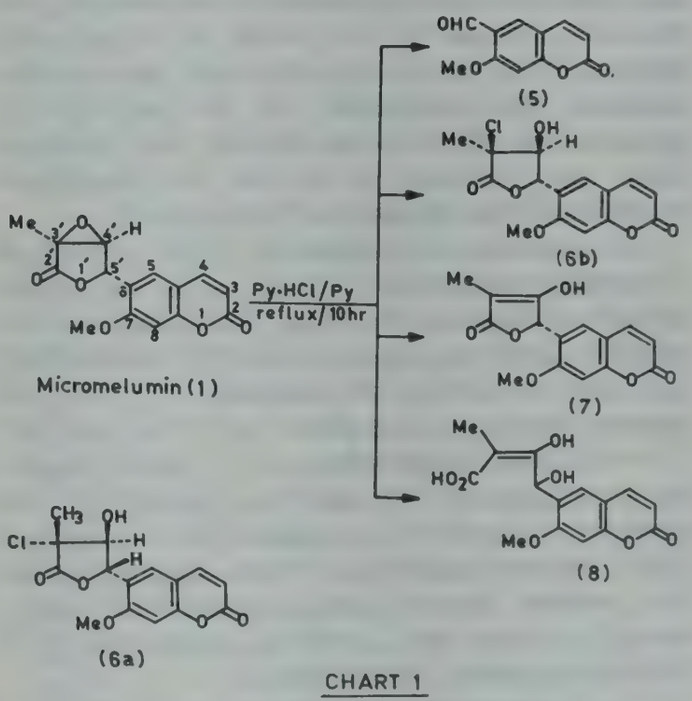

CHART 1

in $CDC1_3$ exhibited the C-3 and C-4 coumarin proton signals at δ 6.28 and 7.66 respectively (*d* each, J = 9.5 Hz), the aromatic C-5 and C-8 protons at 7.54 (*d*, sharply split signal, J = 1.0 Hz) and 6.83 (*s*) respectively, C_7-methoxyl at 3.92 (*s*), the $C_{3'}$-methyl at 1.67 (*s*), and the $C_{4'}$-hydroxyl as a broad signal at 2.69 (disappearing on deuteration), and C-4′ and C-5′ protons appeared at 4.76 (*d*,J = 3.5 Hz) and 6.20 (*dd*, J_1 = 3.5; J_2 = 1.0 Hz) respectively. The coupling constant of 3.5 Hz between the protons at C-4′ and C-5′ confirmed the *trans* coupling. The C-5′ proton underwent further long-range coupling (J = 1.0 Hz) with the C-5 proton. The ^{13}C NMR data (100

†Part V, Banerji J, Das A K & Das B, *Chemy Ind* (In press) (1987).

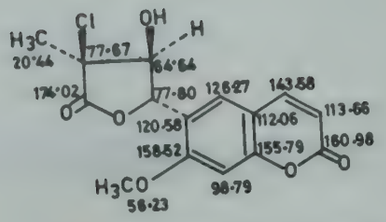

Fig. 1 – ^{13}C NMR data of (6b)

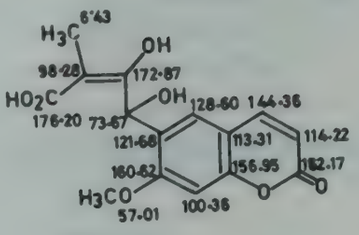

Fig. 2 – ^{13}C NMR data of (8)

MHz in $CDCl_3$) (Fig.1) was also studied together with the DEPT experiment to confirm structure **6b**. At this stage two structures could be proposed for the compound. Compound **6a** had been earlier obtained[7] by the treatment of **1** with HCl in methanol. Direct comparison revealed the non-identity of the two compounds. The transformation of the chloro compound to **7** and **8** had to be carried out under drastic conditions (treatment with pyridinium chloride in dry pyridine at 115° for 10 hr). The reaction was monitored by tlc and only after five hours appreciable amounts of products **7** (12%) and **8** (10%) could be detected and isolated. This was in conformity with the *anti* disposition (not periplanar) of the participating groups as in **6b**. Had the chloride and hydrogen been *syn* periplanar as in **6a** the elimination would have been expected to be more facile.

The structure of compound **7** was settled as 7-methoxycoumarinyl-6-tetronic acid derivative from its detailed spectral data. The compound exhibited significant signals in its 400 MHz PMR spectrum in $CDCl_3$. The coumarin C-3 and C-4 protons appeared as doublets at δ 6.31 and 7.62 (J = 9.7 Hz) respectively and C-5 and C-8 protons as singlets at 7.40 and 6.81 respectively. The methoxy group at C-7 appeared as a singlet at 3.83, and the singlet at 5.65 was assigned to the C-5′ proton. The vinyl methyl appeared as a singlet at δ1.67 and the $C_{4'}-OH$ as a singlet at 10.48 which disappeared on deuteration.

The structure of compound **8** was settled on the basis of its 400 MHz spectrum in acetone-d_6 which displayed signals for coumarin C-3 and C-4 protons as doublets at δ 6.24 and 7.92 respectively (J = 9.5 Hz). The aromatic C-5 and C-8 protons appeared as signlets at δ 7.46 and 7.01 respectively, C-7 methoxy at 4.00 (*s*), C-2′ vinyl methyl at 1.73 (*d*,J = 1.3 Hz) and the C-4′ protons at 6.06 (*d*,J = 1.3 Hz). The presence of homo-allylic coupling of 1.3 Hz between the C-2′ methyl and C-4′ protons and the ^{13}C NMR data (100 MHz in d_6-acetone) (Fig.2) were important in confirming the structure of the product as **8**.

Earlier investigators had reported the isolation of two diastereoisomeric coumarins, minumicrolin (**9**)[8] and murrangatin (**10**)[9,10]. The hemi-synthesis

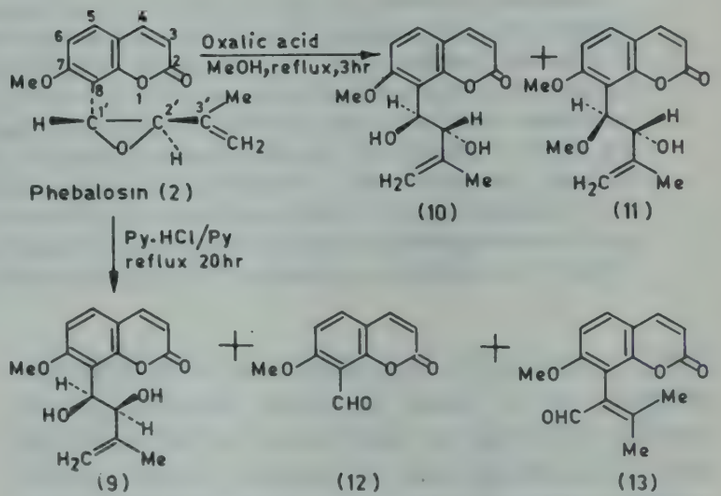

CHART 2

of **9** and **10** has now been carried out starting from phebalosin (**2**) (Chart 2). Phebalosin (**2**) on treatment with methanolic oxalic acid afforded the kinetically controlled *erythro*-isomer murrangatin (**10**), in addition to 1′-methylmurrangatin (**11**). It was felt that if the opening of the epoxide ring could be brought about in a *cis* manner then **9** would be formed. With pyridinium chloride in dry pyridine at 115°, the thermodynamically stable minumicrolin (*threo*-isomer) (**9**) was obtained together with 8-formyl-7-methoxycoumarin (**12**) and 7-methoxy-8-(1′-formyl-2′-methyl prop-1′-enyl) coumarin (**13**) (≡naturally occurring murralongin)[11,12] (Chart 2). The most important features in the PMR spectra of the diastereoisomers was the C-2′ proton which could be utilised to distinguish between such systems. In minumicrolin (**9**) the C-1′ and C-2′ protons appeared as well characterised doublets at δ 5.42 and 4.52 respectively (J = 8.0 Hz) while in murrangatin (**10**) the C-2′ proton was partially hidden under the broadened C-4′ proton singlet (2H) at 4.66. The C-1′ proton in **10** appeared as a doublet at δ 5.32 (J = 8.5 Hz) which sharpened considerably on deuteration.

7-Methoxy-8-formylcoumarin (**12**) was characterised from its spectral properties. Its 80 MHz PMR spectrum in $CDCl_3$ exhibited the C-8 formyl at δ 10.60 (1H, *s*), C-7 methoxyl at 3.99 (3H, *s*), *ortho*-coupled C-5 and C-6 protons at 7.58

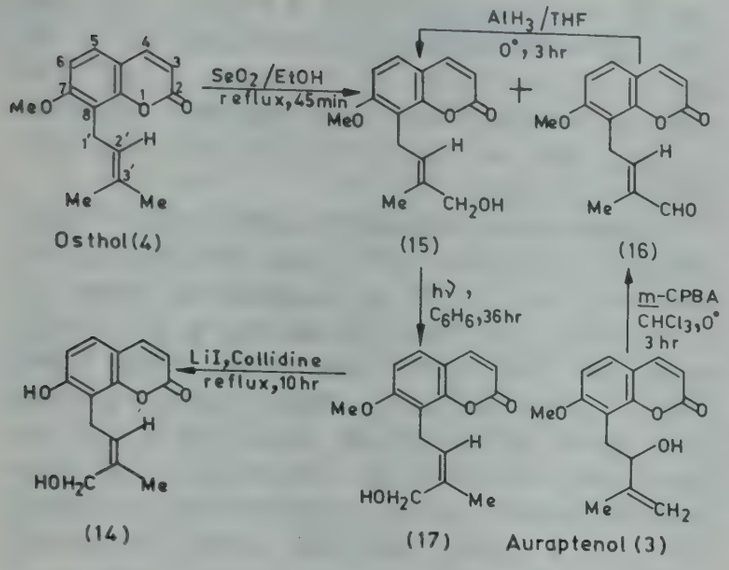

CHART 3

and 6.89 respectively (J = 8.9 Hz) and the C-3 and C-4 coumarin doublets (J = 9.6 Hz) at 6.29 and 7.59.

7-Methoxy-8-(1′-formyl-2′-methylprop-1′-enyl) coumarin (**13**) was identified from its spectral properties and comparison with an authentic sample[13].

Osthol (**4**) and auraptenol (**3**) are two intersting systems which could be utilised for the hemisynthesis of the naturally occurring arnottinin (**14**)[14] (Chart 3). Osthol (**4**) on oxidation with selenium dioxide in ethanol at 78° for 45 min afforded the *E*-methylarnottinin (**15**), along with 7-methoxy-8-(3′-formyl but-2′-enyl)coumarin (**16**). The latter was also obtained by the oxidation of auraptenol (**3**) with *m*-chloroperbenzoic acid at 0° for 3 hr. The aldehyde **16** could be quantitatively converted into *E*-methylarnottinin (**15**) by reduction with aluminium hydride in tetrahydrofuran at 0° for 3 hr. The *E*-isomer (**15**) on irradiation with a medium pressure mercury vapour lamp for 36 hr in benzene at room temperature afforded the *Z*-isomer (**17**), $C_{15}H_{16}O_4$ (M^+ 260), m.p. 110° (benzene), yield 82%. The two isomers could be distinguished by the C-2′ proton signals in their PMR spectra. The C-2′ proton in the *E*-isomer appeared at δ 5.50 (t,J = 7.0 Hz) while that in *Z*-isomer at 5.23 (t,J = 7.0 Hz). Demethylation of **17** using lithium iodide in collidine at reflux temperature led to arnottinin (**14**), which was identified from its spectral data as well as by comparison with an authentic sample.

Experimental Procedure

Melting points were recorded on a Kofler block apparatus and are uncorrected. The UV spectra (in 95% aldehyde-free ethanol) were recorded on a Varian 634 spectrophotometer, IR spectra (KBr) on a Perkin-Elmer 782 spectrophotometer, PMR spectra (in $CDCl_3$ and d_6-acetone using TMS as internal standard) on Varian CFT 20 (80 MHz), JEOL (100 MHz) and Bruker WH-400 (400 MHz) spectrometers, ^{13}C NMR spectra on a Bruker WH-100 spectrometer and the mass spectra on a 70 eV Hitachi RMU 6L mass spectrograph. The yields have been calculated on the basis of the purified products isolated.

Reaction of micromelumin (**1**) *with pyridinium chloride*

To a solution of pyridinium chloride (7 g) in pyridine (10 ml), **1** (500 mg) was added. The reaction mixture was refluxed for 10 hr, cooled, poured into ice-cold water (50 ml), kept for 1 hr and extracted with ether (3×50 ml). The organic layer was washed successively with 1*N* HCl (3×20 ml) and water (3×50 ml), dried and the solvent removed. The crude residue was chromatographed over silica gel. The benzene eluate afforded angelical (**5**), m.p. 254° (benzene) yield 17% (Found: C, 65.2; H, 3.6. Calc for $C_{11}H_8O_4$: C, 64.7; H, 3.9%), identical with an anthentic natural sample.

The benzene-ethyl acetate (9:1) eluate afforded **6b** as a white solid which crystallised from benzene, m.p. 227-28°, yield 25% (Found: C, 55.8; H, 3.8. $C_{15}H_{13}O_6Cl$ requires C, 55.6; H, 4.0%); UV: 325, 251, 221 and 207 nm (log ε 4.14, 3.81, 4.28 and 4.37); IR: 3430, 1790, 1710, 1620, 1500, 1140, 1100, 820 cm^{-1}; MS: *m/z* 326 and 324 (M^+), 296, 288, 272, 244, 229, 205.

The tetronic acid derivative **7** was eluated with benzene - ethyl acetate (4:1) and crystallised from benzene, m.p. 194°, yield 20% (Found: C, 62.2; H, 3.7. $C_{15}H_{12}O_6$ requires C, 62.5; H, 4.2%); UV: 325, 254, 221 and 208 nm (log ε 4.07, 3.85, 4.18 and 4.27); IR: 3400, 1760, 1710, 1620, 1560, 1130, 820 cm^{-1}; MS: *m/z* 288 (M^+), 260, 205, 175.

The benzene-ethyl acetate (1:1) eluate afforded **8** which was further purified by crystallisation from acetone m.p. 240°, yield 15% (Found: C, 58.2; H, 5.2. $C_{15}H_{14}O_7$ requires C, 58.8; H, 4.6%); UV: 325, 257, 223, 208 nm (log ε 4.12, 4.18, 4.30 and 4.40); IR: 3390, 1730, 1620, 1560, 1140, 1100, 820 cm^{-1}; MS: *m/z* 306 (M^+), 288, 278, 276, 260, 205, 175.

Reaction of phebalosin (**2**) *with methanolic oxalic acid*

A mixture of **2** (100 mg) dissolved in methanol (25 ml) and oxalic acid (2.5 g) was refluxed for 3

hr. The solvent was removed and the residue purified by preparative TLC using benzene-ethyl acetate (1:1). Murrangatin (**10**) and 1′-methylmurrangatin (**11**) were separated. Murrangatin (**10**) and 1′-methylmurrangatin (**11**) were separated. Murrangatin (**10**) was crystallised from benzene m.p. 132°, yield 30% (Found: C, 65.7; H, 6.4. $C_{15}H_{16}O_5$ requires C, 65.2; H, 5.8%); UV: 321, 259 and 211 nm (log ε 4.13, 4.00 and 4.37); IR: 3440, 1730, 1610, 1560, 1400, 1120, 1090, 910 cm^{-1}; PMR ($CDCl_3$): δ 7.62 (1H, *d*,*J* = 9.5 Hz, C_4-H), 6.24 (1H, *d*,*J* = 9.5 Hz, C_3-H), 7.39 (1H, *d*,*J* = 8.6 Hz, C_5-H), 6.86 (1H, *d*,*J* = 8.6 Hz, C_6-H), 5.32 (1H, *d*,*J* = 8.0 Hz, $C_{1'}$-H), 4.54 (1H, *d*,*J* = 8.0 Hz, $C_{2'}$-H), 4.66 (2H, *s*, CH_2), 3.96 (3H, *s*, OCH_3), 1.77 (3H, *s*, CH_3); MS: *m/z* 276 (M^+), 258, 242, 227, 211, 205, 203, 175, 162.

1′-Methylmurrangatin (**11**) was purified by crystallisation from benzene, m.p. 120-21°, yield 40% (Found: C, 65.7; H, 6.5. $C_{16}H_{18}O_5$ requires C, 66.2; H, 6.2%); UV: 322, 259, 249, 209 nm (log ε 4.15, 3.67, 3.67, and 4.37); IR: 3460, 1728, 1600, 1560, 1090, 800 cm^{-1}; PMR ($CDCl_3$): δ 7.56 (1H, *d*,*J* = 9.0 Hz, C_4-H), 6.18 (1H, *d*,*J* = 9.0 Hz, C_3-H), 7.34 (1H, *d*,*J* = 8.5 Hz, C_5-H), 6.79 (1H, *d*,*J* = 8.5 Hz, C_6-H), 5.0 (1H, *d*,*J* = 8.0 Hz, $C_{1'}$-H), 4.84 (1H, *d*,*J* = 8.0 Hz, $C_{2'}$-H), 4.60 (2H, *m*, CH_2), 3.84 (3H, *s*, C_7-OCH_3), 3.26 (3H, *s*, C_1-OCH_3), 2.80 (1H, bs, disappeared on deuteration, -OH), 1.62 (3H, *s*, CH_3); MS: *m/z* 290 (M^+), 262, 219, 210, 205, 190, 180, 160.

Reaction of phebalosin (**2**) *with pyridinium chloride*

To a solution of pyridinium chloride (4 g) in pyridine (10 ml), **2** (200 mg) was added. The mixture was refluxed for 24 hr, cooled, poured into ice-cold water (50 ml), for 1 hr and extracted with ether (3 × 50 ml). The extract was washed successively with ice-cold 10% HCl (3 × 20 ml) saturated aq $NaHCO_3$ and water (3 × 50 ml) dried and the solvent removed. The crude residue was purified by preparative TLC using benzene-ethyl acetate (5:1). Three compounds, minumicrolin (**9**), 7-methoxy-8-(1′-formyl-2′-methyl prop-1′-enyl) coumarin (**13**) and 7-methoxy-8-formyl coumarin (**12**) were separated and crystallised from benzene.

Minumicrolin (**9**), m.p. 132°, yield 28% (Found: C, 64.5; H, 5.5. $C_{15}H_{16}O_5$ requires C, 65.2; H, 5.8%); UV: 316, 289, 264, 226 nm (log ε 4.04, 3.90, 3.81 and 4.65; IR: 3420, 1710, 1610, 1560, 1255, 1130, 1100, 840; PMR ($CDCl_3$): δ 7.62 (1H, *d*,*J* = 9.5 Hz, C_4-H), 6.24 (1H, *d*,*J* = 9.5 Hz, C_3-H), 7.40 (1H, *d*,*J* = 8.5 Hz, C_5-H), 6.88 (1H, *d*,*J* = 8.5 Hz, C_6-H), 5.42 (1H, *d*,*J* = 8.0 Hz, $C_{1'}$-H), 4.52 (1H, *d*,*J* = 8.0 Hz, $C_{2'}$-H), 4.99 (2H, *m*, = CH_2), 3.94 (3H, *s*, OCH_3), 1.88 (3H, *s*, CH_3); MS: *m/z* 276 (M^+), 258, 242, 211, 205, 203, 189, 175, 162.

7-Methoxy-8-(1′-formyl-2′-methyl prop-1′-enyl) coumarin (**13**), m.p. 131°, yield 37% (Found: C, 69.2; H, 5.9. $C_{15}H_{14}O_4$ requires C, 69.8; H, 5.4%); UV: 322, 236, 211 nm (log ε: 4.11, 4.12, 4.26); IR: 1722, 1652, 1600, 1560, 1490, 1460, 1365, 1138, 830 cm^{-1}; PMR ($CDCl_3$): δ 7.55 (1H, *d*,*J* = 9.5 Hz, C_4-H), 6.16 (1H, *d*,*J* = 9.5 Hz, C_3-H), 7.40 (1H, *d*,*J* = 8.5 Hz, C_5-H), 6.84 (1H, *d*,*J* = 8.5 Hz, C_6-H), 10.17 (1H, *s*, CHO), 3.78 (3H, *s*, OCH_3), 1.74 (1H, *s*, CH_3), 2.38 (1H, *s*, CH_3). 8-Formyl-7-methoxycoumarin (**12**), m.p. 207°, yield 15% (Found: C, 64.2; H, 4.4. $C_{11}H_8O_4$ requires C, 64.7; H, 3.9%); UV: 326, 310, 236, 211 nm (log ε 4.06, 4.06, 3.86 and 4.30); IR: 1720, 1690, 1590, 1550, 1440, 1110, 1098, 840 cm^{-1}; MS; *m/z* 204 (M^+), 176, 161, 147, 118.

Reaction of osthol (**4**) *with selenium dioxide*

A mixture of osthol (**4**) (500 mg) and freshly sublimed selenium dioxide (225 mg) dissolved in ethanol (70 ml) was refluxed for 45 min, cooled and filtered. Solvent was removed from the filtrate and the residue taken in ether (100 ml), washed with water (3 × 50 ml) and dried. The solution was concentrated and the residue chromatographed over silica gel. Benzene-ethyl acetate (9:1) eluate afforded a white solid which was crystallised from benzene to yield 7-methoxy-8-(3′-formyl but-2′-enyl)coumarin (**16**), m.p. 121°, yield 22% (Found: C, 69.1; H, 5.1. $C_{15}H_{14}O_4$ requires C, 69.8; H, 5.4%); UV: 321, 217 and 207 nm (log ε 4.13, 4.36 and 4.01); IR: 1725, 1680, 1610, 1570, 1500 cm^{-1}; PMR ($CDCl_3$): δ 7.66 (1H, *d*,*J* = 9.5 Hz, C_4-H), 6.25 (1H, *d*,*J* = 9.5 Hz, C_3-H), 7.37 (1H, *d*,*J* = 8.0 Hz, C_5-H), 6.86 (1H, *d*,*J* = 8.0 Hz, C_6-H), 3.90 (2H, *d*,*J* = 6.2 Hz, $C_{1'}$-H), 6.50 (1H, *t*,*J* = 6.2 Hz, $C_{2'}$-H), 9.36 (1H, *s*, CHO), 3.95 (3H, *s*, OCH_3), 1.95 (1H, *s*, CH_3): MS: *m/z* 258 (M^+), 229, 213, 199, 189, 176, 159, 131, 115.

Benzene-ethyl acetate (3:1) eluate gave *E*-methylarnottinin (**15**), which crystallised from benzene, m.p. 110°, yield 55% (Found: C, 68.5; H, 6.5. $C_{15}H_{16}O_4$ requires C, 69.2; H, 6.2%); UV: 322, 257, 251, 215 nm (log ε 4.16, 3.68, 3.64 and 4.22); IR: 3420, 1700, 1610, 1560, 1500, 1110, 1100, 820 cm^{-1}; PMR ($CDCl_3$): δ 7.64 (1H, *d*,*J* = 9.5 Hz, C_4-H), 6.24 (1H, *d*,*J* = 9.5 Hz, C_3-H), 7.32 (1H, *d*,*J* = 8.5 Hz, C_5-H), 6.84 (1H, *d*,*J* = 8.5 Hz, C_6-H), 3.58 (2H, *d*,*J* = 7.0 Hz, $C_{1'}$-

H_2), 5.50 (1H, *t*,*J* = 7.0 Hz, C_2-H), 3.93 (2H, *s*, CH_2OH), 3.88 (3H, *s*, OCH_3), 1.88 (3H, *s*, CH_3).

Oxidation of auraptenol (**3**) *with m-chloroperbenzoic acid*

To a solution of **3** (100 mg) in dry chloroform (20 ml) was added *m*-chloroperbenzoic acid (80 mg) and the reaction mixture stirred at 0° under nitrogen atmosphere for 3 hr. The mixture was diluted with water (20 ml), chloroform layer separated, washed successively with aq soldium bisulphite (3×20 ml), aq sodium bicarbonate (3×20 ml) and water (3×20 ml) and dried. Upon concentration a white solid was obtained which was purified by crystallisation from benzene to yield **16**, m.p. and m.m.p. 121°, yield 45%.

Reduction of 7-methoxy-8-(3'-formylbut-2'-enyl) coumarin (**16**) *with aluminium hydride*

To a well-stirred ice-cold mixture of aluminium hydride (25 ml) prepared by the method of Brown *et al.*[15] and THF (10 ml), was added **16** (500 mg) in THF (10 ml) rapidly under nitrogen atmosphere. After 3 hr methanol (50 ml) was added, filtered and the filtrate concentrated. The residue was chromatographed over silica gel. The benzene-ethyl acetate (3:1) eluate afforded *E*-methylarnottinin (**15**), m.p. and m.m.p. 110° (benzene), yield 85%.

Ultraviolet irradiation of E-methylarnottinin (**15**)

E-Methylarnottinin (**15**) (50 mg) dissolved in dry benzene (100 ml) was irradiated with a medium pressure mercury vapour lamp (wave lengths 313 and 336 nm) for 36 hr. The solvent was evaporated and the residue on chromatographic resolution over silica gel afforded *Z*-methylarnottinin (**17**) in the benzene eluate, m.p. 110° (benzene), yield 82% (Found: C, 69.7; H, 6.4. $C_{15}H_{16}O_4$ requires, C, 69.2; H, 6.2%); PMR ($CDCl_3$): δ 7.64 (1H, *d*,*J* = 9.5 Hz, C_4-H), 6.26 (1H, *d*,*J* = 9.5 Hz, C_3-H), 7.32 (1H, *d*,*J* = 8.5 Hz, C_5-H), 6.86 (1H, *d*,*J* = 8.5 Hz, C_6-H), 3.59 (2H, *d*,*J* = 7.0 Hz, C_1-H_2), 5.23 (1H, *t*,*J* = 7.0 Hz, C_2-H), 4.42 (2H, *s*, CH_2OH), 3.93 (3H, *s*, OCH_3), 1.84 (3H, *s*, CH_3).

Demethylation of Z-methylarnottinin (**17**)

A mixture of **17** (50 mg) and lithium iodide (50 mg) dissolved in dry collidine (10 ml) was refluxed under nitrogen atmosphere for 10 hr. The mixture was cooled, poured into water (20 ml), extracted with chloroform (3×20 ml), the chloroform layer washed successively with chilled 1*N* HCl (3×20 ml), 2% aq sodium bicarbonate and water (3×20 ml) and dried. The solution was concentrated when a solid was obtained. This was purified by crystallisation from benzene to yield arnottinin (**14**), m.p. 195°, yield 12% (Found: C, 67.4; H, 5.9. $C_{14}H_{14}O_4$ requires C, 68.3; H, 5.7%); UV: 330 and 259 nm (log ε 4.16 and 3.65); IR: 3360, 3140, 1700, 1610, 1580, 1510, 1450 cm^{-1}; PMR (CD_3COCD_3): δ 7.96 (1H, *d*,*J* = 9.5 Hz, C_4-H), 6.14 (1H, *d*,*J* = 9.5 Hz, C_3-H), 7.40 (1H, *d*,*J* = 8.5 Hz, C_5-H), 6.88 (1H, *d*,*J* = 8.5 Hz, C_6-H), 9.52 (1H, *s*, disappeared on deuteration -OH), 3.60 (2H, *d*,*J* = 7.0 Hz, C_1-H_2), 5.32 (1H, *t*,*J* = 7.0 Hz, C_2-H), 4.34 (2H, *s*, CH_2OH), 1.72 (3H, *s*, CH_3).

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Synthesis & Stereochemistry of Some 2,6-Diaroyl-3,5-diaryltetrahydro-1,4-thiazine-1,1-dioxides

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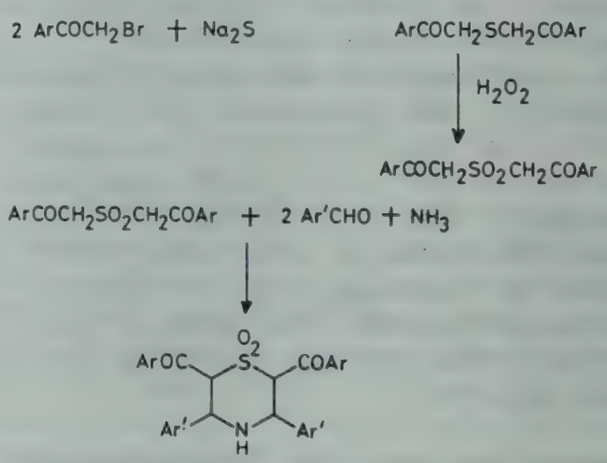
2,6-Diaroyl-3,5-diaryltetrahydro-1,4-thiazine-1,1-dioxides have been synthesized and their stereochemistry has been investigated using PMR data. These compounds adopt chair conformation and the substituents are equatorially oriented except in the case of 3,5-bis(*o*-methoxyphenyl) compound which has an aroyl group in the axial-position. The aryl groups without *ortho* or *meta* - substituents rotate freely. However, *ortho* and *meta*-substituted aryl groups seem to adopt fixed conformations.

Previously[1], 3,5-diaryltetrahydro-1,4-thiazine-1,1-dioxides were synthesized by condensing sulphonyldiacetic acid with aromatic aldehydes and ammonia and their stereochemistry was elucidated using dipole moments and PMR spectra[2]. We now report the synthesis of 2,6-diaroyl-3,5-diaryltetrahydro-1,4-thiazine-1, 1-dioxides (**1-8**) by the condensnation of aromatic aldehydes and ammonia with bis(aroylmethyl) sulphones, which were prepared starting from appropriate ω-bromoacetophenones as shown in Scheme 1.



	Ar	Ar′
1	Ph	p-tolyl
2	Ph	m-tolyl
3	Ph	o-ClC_6H_4
4	Ph	o-$OMeC_6H_4$
5	Ph	m-$NO_2C_6H_4$
6	p-ClC_6H_4	o-ClC_6H_4
7	p-ClC_6H_4	p-tolyl
8	p-ClC_6H_4	m-$NO_2C_6H_4$

Scheme 1

The physical data of these compounds are given in Table 1. Their PMR spectra† were recorded at 270 MHz and the spectral data are furnished in Table 2.

Compounds other than **4** exhibit two doublets for the heterocyclic protons. The doublet at 6.2-6.5 is due to the protons at C-2 and C-6 and that at 4.7-5.3 is due to the protons at C-3 and C-5. This assignment is made by comparing the chemical shifts of **1** and **3**. The *o*-chloro subsituent is known to cause deshielding of the benzylic protons by about 0.6 ppm[3]. For **1** the doublets are at

Table 1 — Physical Data of Compounds 1-8

Compd	Yield (%)	m.p. °C	Mol. formula	Found (%)		Calc. (%)	
				C	H	C	H
1	90	206	$C_{32}H_{29}O_4NS$	73.5	5.4	73.4	5.6
2	88	207	$C_{32}H_{29}O_4NS$	73.1	5.3	73.4	5.6
3	85	196	$C_{30}H_{23}O_4NSCl_2$	63.6	4.0	63.8	4.1
4	75	199	$C_{32}H_{29}O_6NS$	69.3	5.2	69.2	5.3
5	80	223	$C_{30}H_{23}O_8N_3S$	61.2	3.6	61.5	4.0
6	95	196	$C_{30}H_{21}O_4Cl_4NS$	56.8	3.1	56.9	3.3
7	90	218	$C_{32}H_{27}O_4Cl_2NS$	64.9	4.6	64.9	4.6
8	95	212	$C_{30}H_{21}O_8Cl_2N_3S$	55.4	3.1	55.1	3.2

†Chemical shifts in δ, ppm throughout the paper.

Table 2—PMR Spectral Data of Compounds 1-8

[Chemical shifts in δ, ppm]

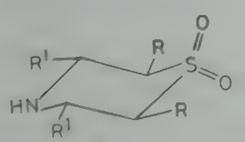
Compd	Ar-*H*	C_2-H and C_6-H	C_3-H and C_5-H	NH	Other protons
1	7.97(*m*, 4H); 7.67(*m*, 2H); 7.56(*m*, 4H); 7.32(*m*, 4H); 7.04(*m*, 4H)	6.32(*d*, 2H; *J*= 10 Hz)	4.79(*d*, 2H; *J*= 10 Hz)	2.3	2.1(Me)
2	7.97(*m*, 4H); 7.67(*m*, 2H); 7.56(*m*, 4H); 7.26(*s*, 2H); 7.20(*dd*, 2H); 6.96(*dd*, 2H)	6.36(*d*, 2H; *J* = 10.3 Hz)	4.77(*d*, 2H; *J* = 10.2 Hz)	2.3	2.1(Me)
3	7.97(*m*, 4H); 7.67(*m*, 2H); 7.56(*m*, 4H); 7.44(*m*, 4H); 7.20(*m*, 4H)	6.54(*d*, 2H; *J* = 10 Hz)	5.39(*d*, 2H; *J* = 10 Hz)	2.4	
4	6.8 - 8	6.42(*d*, 1H; *J* = 10 Hz) 5.79(*d*, 1H; *J* = 3 Hz)	5.15(*d*, 1H)* 4.98(*d*, 1H)	2.4	4.0 and 3.9(OMe)
5	8.4(*s*, 2H); 7.4-8.1(*m*, 16 H)	6.54(*d*, 2H; *J* = 10 Hz)	5.08(*d*, 2H; *J* = 10 Hz)	2.5	
6	8.06(*m*, 4H); 7.72(*m*, 4H) 7.4(*m*, 4H); 7.2(*m*, 4H)	6.50(*d*, 2H; *J* = 10 Hz)	5.34(*d*, 2H; *J* = 10 Hz)	2.5	
7	7.98(*m*, 4H); 7.66(*m*, 4H) 7.3(*m*, 4H); 7.05(*m*, 4H)	6.29(*d*, 2H; *J* = 10 Hz)	4.75(*d*, 2H; *J* = 10 Hz)	2.5	2.1(Me)
8	8.42(*s*, 2H); 7.4 - 8.1(*m*, 16 H)	6.49(*d*, 2H; *J* = 10 Hz)	5.04(*d*, 2H; *J* = 10 Hz)	2.5	

*Signals were broad and the coupling constants could not be determined accurately

4.79 and 6.32 whereas for **3** they are at 5.39 and 6.54. Obviously, the peaks at 4.79 and 5.39 must be due to benzylic protons.

The coupling constant between C-2 and C-3 protons is about 10 Hz indicating a diaxial coupling. Hence, compounds **1,2,3,5,6,7** and **8** adopt chair conformation (**9**) with all the substituents in the equatorial positions.

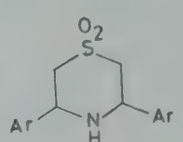
In 3,5-diaryltetrahydro-1,4-thiazine-1-1-dioxides (**10**) the methylene protons absorb at 3.1-3.2 (ref.2). Hence, the aroyl group deshields the protons at C-3 and C-6 by about 3 ppm. The deshielding effect of an aroyl group in acyclic molecules may be determined by comparing the chemical shifts of methyl protons in *n*-alkanes and the chemical shifts of methylene protons in $PhCOCH_2R$. The methyl protons in *n*-alkanes generally absorb around 0.9-1.1 (ref. 4) whereas methylene protons in $PhCOCH_2R$ absorb around 2.9 (ref.4). Thus, it is seen that in acyclic molecules the deshielding produced by an aroyl group on an adjacent proton is about 2 ppm. Hence, the deshielding effect of the aroyl group on the methine proton in compounds **1,2,3,5,6,7** and **8** is considerably larger than that observed in acyclic molecules. This may be explained as follows:



9

R ArCO

R¹ Ar



10

In acyclic molecules the benzoyl group can adopt various possible conformations around the CO-C bond and each of the adjacent protons experience an average magnetic anisotropic effect of the carbonyl group. In compounds **1,2,3,5,6,7** and **8** the aroyl group is probably oriented as shown in Fig.1 so that the methine proton experiences fully the deshielding caused by the magnetic anisotropy of the carbonyl group.

Compound **4** exhibits four doublets, each corresponding to one proton, for the heterocyclic protons. The doublets at 6.42 and 5.79 can be conveniently assigned to C-2 and C-6 protons. The doublet at 5.79 has a *J* value of 3 Hz and that at 6.42 has a *J* value of 10 Hz. This suggests that in **4** one aroyl group is axially oriented so that the vicinal coupling is only J_{ae}. The appearance of the proton at the axially substituted carbon at a higher field may be due to a lesser deshielding by the carbonyl group.

Compound **1** shows five distinct signals for the aromatic protons. The signal at 7.97 is due to *ortho*-protons of the benzoyl group. These are deshielded by the electronic and magnetic effects of the carbonyl group. The signals at 7.67 and 7.56 can be assigned, respectively, to *para*- and *meta*-protons of the benzoyl group. The *meta*-protons of *p*-tolyl group (*meta* with respect to the benzylic carbon) must be expected to absorb near a position where benzene protons absorb since these protons are far away from the carbonyl group and the nitrogen lone pair. Therefore, the signal at 7.32 can be assigned to these protons. The *ortho*-

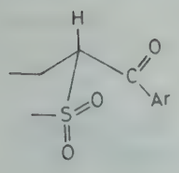


FIG.1

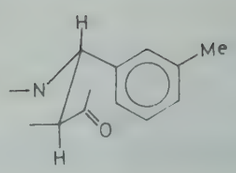


FIG. 2

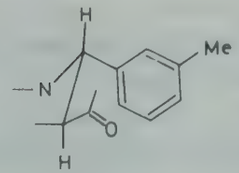


FIG. 3

protons of the *p*-tolyl group are shielded since they lie above the plane of the carbonyl group (Fig.2) and appear at 7.04. The shielding by the carbonyl group, obviously, outweighs the deshielding effect of the nitrogen lone pair[2,3,5].

Compound **7** behaves similar to **1** except in the absence of the signal due to the *para*-protons of the benzoyl group.

Moreover, the *ortho*-protons are chemically equivalent in **1** and **7**. The *meta*-protons also are chemically equivalent in these compounds. This suggests that the aryl groups rotate freely under the experimental conditions.

In **2**, though the absorbance of the benzoyl protons is exactly as in **1**, four distinct signals appear for the aromatic protons of the *m*-tolyl group. The singlet at 7.26 is due to the *o,o*-proton (*ortho* to the benzylic carbon as well as to the methyl group). The signal at 7.4 has a width of about 15 Hz indicating two *ortho*-couplings and hence must be due to the *p,m*-proton. The signal at 6.96 must be due to the *o,p*-proton. Among the two *ortho*-protons, the one which is *para* to the methyl group absorbs at a higher field than that which is *ortho* to the methyl group. This suggests that the *m*-tolyl group is preferably oriented such that the methyl group is *anti* to the carbonyl group as shown in Fig.3.

For **3** and **6** only two signals of equal area are observed for the protons of *o*-chlorophenyl group. Obviously, there are only two sets of chemically different protons in this group. This is a case of accidental chemical equivalence.

In the *m*-nitrophenyl compounds **5** and **8** the singlet at 8.4 is due to the *o,o*-proton. The spectrum for the other aromatic protons appears very complex. In **4**, the aromatic protons absorb in a wide range suggsting inequality of the two phenyl as well as the two *o*-methoxyphenyl groups.

The appearance of two signals for the methoxy protons also supports the axial-orientation of one of the aroyl groups. The reason for the presence of one of the aroyl groups in the axial position in **4** is not clear.

Experimental Procedure

PMR spectra were recorded on a Brucker WH-270 NMR spectrometer at 270 MHz operating at a field of 6.35 tesla. Samples were prepared by dissolving 50 mg material in 0.5 ml of DMSO-d_6 containing 1% TMS.

Phenacyl bromide was prepared according to the procedure of Cowper and Davidson[6]. This was converted into diphenacyl sulphide by treating with Na_2S following the procedure of Tafol and Martiz[7]. The sulphide was oxidised with H_2O_2 using the procedure of Fromm and Flaschen[8].

2,6-*Diaroyl*-3,5-*diaryltetrahydro*-1,4-*thiazine*-1,1-*dioxides* (**1-8**)

A mixture of diphenacyl sulphone (0.01 mol), an aldehyde (0.02 mol) and ammonium acetate (0.01 mol) in ethanol (25 ml) was refluxed for 15 min. The solid that separated on cooling was collected and recrystallised either from dioxane (compounds **1-5**) or acetic acid (compounds **6-8**).

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Conformational Analysis of 1,1,2-Trisubstituted Ethanes by PMR: Part I – 3-Aryl-2-methylpropanols

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A self-consistent model for the conformational analysis of 1,1,2-trisubstituted ethanes is developed, based on the temperature dependence of vicinal proton-proton coupling constants. Synthesis of racemates of 2(*R*), 3(*S*)- and 2(*S*), 3(*R*)-2,3-dideutero-2-methyl-3-phenyl-1-propanols is accomplished using known reactions of well-defined stereochemistry. By comparing the PMR spectra of undeuterated and deuterated compounds, an unequivocal assignment of benzylic protons is made. Concentration and variable-temperature studies have revealed the existence of very weak intramolecular interaction in three alcohols, *p*-R – C_6H_4 – CH_2 – CHMe – CH_2 – OH, where R = NO_2, H and OMe.

The primary aim of the present work is to establish the conformational stability of amino acids. As a first step, a self-consistent model for conformational analysis of 1,1,2-trisubstituted ethanes is developed, based on the Karplus relation for vicinal coupling constant as a function of the dihedral angle. A salient feature of this model is the estimation of the proportionality factor, based on the observed vicinal coupling constants.

An unequivocal assignment of the methylene protons is accomplished by a stereospecific deuteration. Effects of solvents and concentration on conformational free energies have also been evaluated.

In subsequent papers of the series, detailed variable temperature studies on a series of model compounds will be reported, to establish the effects of weak intramolecular interactions on conformational enthalpies and entropies; deviation from staggeredness and its minifestations in observed spectral parameters as a function of temperature will also become apparent. These informations will then be applied in the reliable conformational analysis of some amino acids.

Materials and Methods

NMR techniques

All NMR spectra were recorded on a Varian Associates HA-100 spectrometer, using a Hewlett-Packard model 200 AB audio oscillator for double resonance experiments. Most spectra were recorded in the frequency sweep mode, the solvent signal (e.g. chloroform) being used as a field-frequency lock. Frequency calibrations were done using the sweep oscillator and frequency counter.

For temperature calibrations, methanol and ethylene glycol peaks were decoupled by adding 0.03% hydrochloric acid, and these samples were standardized against Varian standards on the A-60 spectrometer. The hydroxyl peak in each case was recorded at 50 Hz sweep width, sweep frequency determined by interpolation, and manual oscillator frequency substracted from this to obtain the chemical shift. The chemical shift was substituted in Van Geet equations[1] to calculate the true temperature.

TMS was used as an internal reference. The samples were deoxygenated and sealed. The crucial benzylic signals were recorded at 100 Hz sweep width, under slow passage conditions. All spectra were analysed LAOCOON2[2].

General

Infrared spectra were recorded either in KBr or as thin films on Beckmann IR-5 or IR-10 spectrometers. Mass spectra were recorded on a Hitachi Perkin-Elmer RMU-60 spectrometer at 70 eV.

2-Methyl-3-phenyl-1-propanol

Benzaldehyde was condensed[3] with propanal to give a product which was fractionally distilled under reduced pressure to afford pure (PMR) α-methylcinnamaldehyde; b.p. 58-61°/1.0-1.2 mm; yield 70%. α-Methylcinnamaldehyde was reduced with lithium aluminium hydride[4] to yield 2-methyl-3-phenylprop-2-en-1-ol, characterized by PMR; b.p. 71-75°/0.5 mm; yield 89%. The above compound was catalytically (Pd/C) reduced to give 2-methyl-3-phenyl-1-propanol; b.p. 68-71°/0.03-0.04 mm; pure by PMR; yield 90%.

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3-(4-Methoxyphenyl)-2-methyl-1-propanol

The Grignard reagent from *p*-bromoanisole was prepared[5], and reacted with 3-chloro-2-methylpropene to give 3-(4-methoxyphenyl)-2-methylprop-1-ene; yield 60%. This compound was subjected to hydroboration[6] to yield 3-(4-methoxyphenyl)-2-methyl-1-propanol, with a satisfactory PMR spectrum; yield 80%.

2-Methyl-3-(4-nitrophenyl)propan-1-ol

α-Methylcinnamic acid was synthesized by the Perkin-type condensation[7] of benzaldehyde with propanoic anhydride; m.p. 74°; pure by PMR; yield 36%. α-Methylcinnamic acid was catalytically (Pd/C) hydrogenated to yield 2-methyl-3-phenylpropanoic acid, which was nitrated[8] to give 2-methyl-3-(4-nitrophenyl)propanoic acid; M^+ 209; yield 67%. The above acid, on diborane reduction[6], yielded 2-methyl-3-(4-nitrophenyl)-1-propanol; pure by PMR; yield 75%.

2,3-Dideutero-2-methyl-3-phenylpropanoic acid

A solution of α-methylcinnamic acid (3g) in ethanol (20 ml) and 20% Pd/C (0.3 g) were placed in a 250-ml filter flask containing a magnetic stirring bar. A balloon was attached to the side-arm and a one-holed rubber stopper carrying a three-way stop-cock to the mouth. The three-way stop-cock was connected to a deuterium tank (The Matheson Co.) and an aspirator. The system was evacuated, flushed with deuterium gas and stirred. The absorption of deuterium was over in about 15 min. The mixture was filtered, ethanol evaporated and the residue distilled under reduced pressure to yield 2,3-dideutero-2-methyl-3-phenylpropanoic acid (2.5 g); pure by PMR; yield 80%.

2,3-Dideutero-2-methyl-3-phenyl-1-propanol: (i) Borohydride reduction of α-methylcinnamaldehyde

To a solution of α-methylcinnamaldehyde (5 g, 0.034 mol) in ethanol (20 ml) was added sodium borohydride (0.6 g, 0.016 mol) in small portions, with stirring. After stirring for 1 hr, excess borohydride was decomposed (dil HCl), the mixture diluted to 300 ml with water and extracted with benzene. Benzene extract was dried (Na_2SO_4), the solvent evaporated and the residue distilled under reduced pressure to yield 4 g (80%) of 2-methyl-3-phenylprop-2-en-1-ol. The PMR spectrum of the product was identical with that of the sample prepared earlier.

(ii) Deuteration of 2-methyl-3-phenylprop-2-en-1-ol

2-Methyl-3-phenylprop-2-en-1-ol (1g) was treated first with sodium borodeuteride (0.2 g; Merck-Sharp and Dohme, Canada) and boron trifluoride etherate (1.2 g), and then with acetic acid-d_1 (3 ml; prepared from acetic anhydride and deuterium oxide), following the procedure described by Brown and Murray[6]. The yield of purified 2,3-dideutero-2-methyl-3-phenyl-1-propanol was 0.6 g (60%).

(iii) Reduction of 2,3-dideutero-2-methyl-3-phenylpropanoic acid

The title acid (1 g) was reduced to the corresponding alcohol, by first treating with sodium borohydride and boron trifluoride etherate, and then with propanoic acid, following a procedure described by Brown and Murray[6]; yield 0.5 g.

Infrared and PMR spectra of samples prepared in steps (ii) and (iii) are identical. An IR absorption at 2140 cm^{-1}, which was very weak in $C_6H_5—CH_2-CDMe-CH_2OH$, increased in intensity in $C_6H_5-CHD-CDMe-CH_2OH$. This absorption was absent in the non-deuterated alcohol and hence must be due to νC − D mode. Another peak at 740 cm^{-1} that was strong in both $C_6H_5-CH_2-CHMe-CH_2OH$ and $C_6H_5-CH_2-CDMe-CH_2OH$ becomes weaker in $C_6H_5-CHD-CDMe-CH_2OH$. The band must be caused by asymmetric bending vibrations.

(iv) Assignment of benzylic protons

By comparing the PMR spectrum of *trans*-cinnamic acid with that of the α-methylcinnamic acid, the latter was ascertained to be the *trans*-isomer (Table 1).

Careful reduction of $C_6H_5-CH=CMe-COOH$, with deuterium gas over palladium catalyst yielded a saturated acid, $C_6H_5-CHD-CDMe-COOH$, which displayed only one signal in the benzylic region, allowing a margin for scrambling.

The above acid was reduced by diborane and acid to yield the alcohol, $C_6H_5-CHD-CDMe-CH_2OH$, which exhibited only one absorption in the benzylic region (Table 1).

Synthetic α-methylcinnamaldehyde was reduced by sodium borohydride to yield the alcohol, $C_6H_5-CH=CMe-CH_2OH$ (Table 1), which was reduced by sodium borodeuteride and boron trifluoride, and then with acetic acid-d_1 to yield the same saturated alcohol.

The two routes are mutually supplementary; the starting material in the former route is known to be *trans* and the reductive step in the latter route is definitely known to be *cis* addition[9]. *Cis* addition of deuterium to pure *trans* compound is ex-

Table 1—Comparison of Chemical Shifts and Assignment of Stereochemistry to 2,3-Dideutero-2-methyl-3-phenyl-1-propanol

δ, ppm	Multiplicity	No. of H	Assignment
(I) CINNAMIC ACID (*trans*)			
13.21	*s*	1	carboxylic
7.83	d(J=16)	1	β-vinylic
7.45	*ss**	5	aromatic
6.46	*d*(J=16)	1	α-vinylic
(II) α-METHYLCINNAMIC ACID (*trans*)			
12.76	*s*	1	carboxylic
7.80	*q*(J=1.4)	1	β-vinylic
7.32	*ss**	5	aromatic
2.11	*d*(J=1.3)	3	methyl
(III) $C_6H_5-CH=CMe-CH_2OH$ (*trans*)			
7.13	*ss**	5	aromatic
6.42	unresolved *q*(J=1.3)	1	vinyl
4.04	unresolved *d*(J=0.6)	2	methylene
3.75 (variable)	*s*	1	hydroxyl
1.80	*d*(J=1.2)	3	methyl
(IV) $C_6H_5-CHD-CDMe-CH_2OH$ (RS+SR)			
7.06	*ss**	5	aromatic
3.34	*bs*	2	methylene
3.14 (variable)	*s*	1	hydroxyl
2.24	*bs*	1	benzylic (high-field)
0.84	*bs*	3	methyl

*structural singlet

pected to yield only the enantiomers *RS* and *SR*, the benzylic protons of which are equivalent. This was found to be the high-field one, and hence the configuration shown in Fig. 1a.

Hence in the compounds under study, a correlation is made between stereochemistry and chemical shift of benzylic protons, as shown in Fig. 1b. This is made use of in the identification of *trans* and *gauche* forms in the conformational analysis that follows.

Treatment of Data

The model: (i) Form of Karplus equation

The original Karplus equation[10].

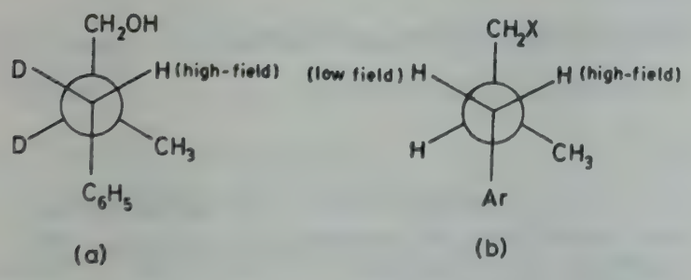


Fig. 1—(a) Correlation between chemical shift and stereochemistry of 2(s), 3(R)-2,3-dideutero-2-methyl-3-phenyl-1-propanol; and (b) assignment of benzylic protons in $Ar-CH_2-CHMe-CH_2-X$.

$$J = \begin{matrix} 8.5\cos^2\phi - 0.28,\ 0° \leqslant \phi \leqslant 90° \\ 9.5\cos^2\phi - 0.28,\ 90 \leqslant \phi \leqslant 180° \end{matrix} \quad (1)$$

and its modification by Williamson and Johnson[11],

$$J = \begin{matrix} 10\cos^2\phi,\ 0° \leqslant \phi \leqslant 90° \\ 16\cos^2\phi,\ 90° \leqslant \phi \leqslant 180° \end{matrix} \quad (2)$$

relate the vicinal coupling constant with $\cos^2$ of the dihedral angle. The same relation is accepted in the present studies. The more recent Karplus relation[12],

$$J = 4.22 - 0.5\cos\phi + 4.5\cos 2\phi \quad (3)$$

is found to exhibit no real advantage in calculations. In addition, it gives low calculated values.

(ii) Ratio of Karplus constants

An examination of the theoretical considerations-Fermi contact at fixed *s* character-reveals that the actual magnitude of the proportionality constants in Eq. (2) depends on the substituents in the ethane system. Hence in the present studies,

the values of the Karplus constants are not specified *a priori*; only the ratio is predetermined, the actual value being determined by the simultaneous equations relating the observed coupling constants.

The equilibrium dihedral angles are approximated to the staggered ones, unless there is compelling evidence to the contrary. The dependence of the ratio of Karplus constants on dihedral angles is further discussed in the section on error analysis.

(*iii*) *Third conformer*

In the compounds under study, the three conformers shown in Fig. 2 are in equilibrium. It is recognized that the *gauche-gauche* form (conformer III) 'can be neglected'[13] in 1,1,2-trisubstituted ethanes. Snyder, who investigated the conformational stability of twelve 1,1,2-trisubstituted ethanes, suggested that 'conformers with two *gauche* interactions are highly disfavoured[14]. In accordance with the foregoing facts, we conclude that the third conformer is the least populated one in our compounds as well.

(*iv*) *Energy difference between conformers II and III*

The conformational energy minima in butane differ by 0.8-0.9 kcal/mol (ref. 15, 16). This instability of the *gauche* form butane is ascribed entirely to the methyl-methyl interaction, taken to be 0.8-0.9 kcal/mol (ref. 17). In agreement, the axial and equatorial forms of methylcyclohexane differ by two such interactions, and the energy difference between them is 1.7 kcal/mol (ref. 18).

Similarly, the two forms of phenylcyclohexane differ by 3.0 kcal/mol (ref. 19). Therefore, in our compounds, where conformers II and III differ by one methyl-phenyl interaction, the energy difference can be approximated to be 1.5 kcal/mol.

The variation of this estimate over ± 0.4 kcal/mol does not change the conformational equilibrium between I and II to any significant extent.

ΔH^0 and ΔS^0 from coupling constants

Based on the knowledge of the absolute stereochemistry of the benzylic protons (Fig. 1b), and on the above approximations, Eqs (4-7) are set up.

$$n_1 J_0 \cos^2\phi_1 + n_2 J_0' \cos^2\phi_3 + n_3 J_0' \cos^2\phi_5 = J_a \quad \ldots (4)$$

$$n_1 J_0' \cos^2\phi_2 + n_2 J_0 \cos^2\phi_4 + n_3 J_0' \cos^2\phi_6 = J_b \quad \ldots (5)$$

$$n_1 + n_2 + n_3 = 1 \quad \ldots (6)$$

$$16 J_0' = 10 J_0 \quad \ldots (7)$$

where ϕ_1, ϕ_3 and ϕ_5 are the dihedral angles between the methine proton and the high-field proton in the three conformers respectively; ϕ_2, ϕ_4 and ϕ_6 between the methine and the low-field one; J_0' is the Karplus constant between 0° and 90°; J_0 between 90° and 180°; n_1, n_2 and n_3 are the molar fractions of the conformers I, II and III, respectively; J_a is the high-field and J_b is the low-field coupling constant.

Solution of these equations provides the conformer populations at each temperature. From these, free energy difference between the conformers at each temperature can be calculated, using Eq. (8),

$$\Delta G^0 = -RT \ln K \quad \ldots (8)$$

where $K = n_2/n_1$.

Equation (8), when substituted in the familiar relation (9)

$$\Delta G = \Delta H - T\Delta S \quad \ldots (9)$$

gives the expression,

$$\ln K = -(\Delta H/RT) + (\Delta S/R) \quad \ldots (10)$$

which shows a linear relationship between $\ln K$ and 1/T. The slope of the linear plot gives the enthalpy and the intercept gives the entropy change.

GIGOS, a computer program

(i) The program starts by assuming the absence of rotamer III, when Eqs (4)-(7) reduce to Eqs (11)-(13)

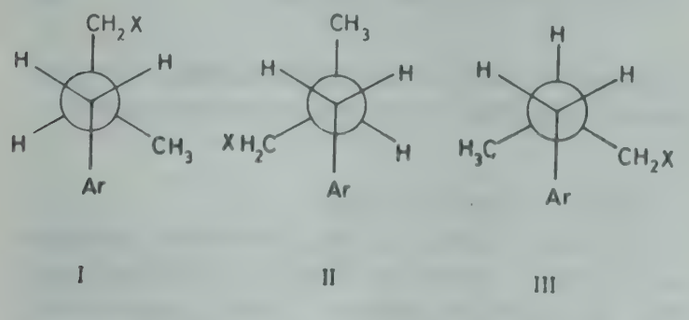
$$16\, j\, n_1 \cos^2\phi_1 + 10\, j\, n_2 \cos^2\phi_3 = J_a \quad \ldots (11)$$

$$10\, j\, n_1 \cos^2\phi_2 + 16\, j\, n_2 \cos^2\phi_4 = J_b \quad \ldots (12)$$

$$n_1 + n_2 = 1 \quad \ldots (13)$$

(ii) The reduction factor, j, is calculated in a crude form from the experimental values at 25°C; using Eq. (14)

$$j = (J_a + J_b)/16\, n_1 \cos^2\phi_1 + 10\, n_2 \cos^2\phi_3 + 10\, n_1 \cos^2\phi_2 + 16\, n_2 \cos^2\phi 4) \quad \ldots (14)$$



I II III

Fig. 2—Three conformers (I-III) of Ar – CH_2 – CHMe – CH_2 – X.

Subsequently, n_1, n_2 and the free energy difference between the two conformers are calculated.

(iii) The estimate of free energy difference at 25° between conformers II and III is used at this stage to calculate the approximate populations of the three conformers.

(iv) The reduction factor, j, in a refined form is then estimated by Eq. (15)

$$J=(J_a+J_b)/(16\ n_1\cos^2\phi_1+10\ n_2\cos^2\phi_3 + 10\ n_3\cos^2\phi_5+10\ n_1\cos^2\phi_2+16\ n_2\cos^2\phi_4 + 10\ n_3\cos^2\phi_6) \quad \ldots (15)$$

Refined values of n_1, n_2 and n_3 are obtained by substituting this in Eqs (4)-(7). The sequence in this step is repeated four times; the convergence is extremely fast.

(v) Karplus constants obtained from this refined j are used in Eqs (4)-(7) to calculate the mol fractions n_1, n_2 and n_3 at each temperature. Free energies for each of the three equilibria at each temperature may be calculated at this stage.

(vi) A standard least square regression analysis[20] is performed finally to linearize (ln K) versus 1/T, and to obtain ΔH, and ΔS values. A standard error analysis is done along with this.

(vii) After the determination of ΔS, enthalpy difference may separately be calculated at each temperature.

The program is written in FORTRAN and was executed on IBM system 360.

Error analysis: (*i*) *Inherent mean square error*

Since $\phi_1 \simeq \phi_4 \simeq 180°$ and $\phi_2 \simeq \phi_3 \simeq \phi_5 \simeq \phi_6 \simeq 60°$,

$$n_1 \simeq (J_a - J_0' \cos^2\phi_2)/(J_0\cos^2\phi_1 - J_0'\cos^2\phi_2) \quad \ldots (16)$$

and

$$n_2 \simeq (J_b - J_o' \cos^2\phi_2)/(J_0\cos^2\phi_1 - J_0'\cos^2\phi_2) \quad \ldots (17)$$

The crucial equilibrium constant, K, then is given by Eq. (18)

$$K = \frac{n_2}{n_1} = \frac{J_b \pm \varepsilon_1 - J_0' \cos^2\phi_2}{J_a \pm \varepsilon_2 - J_0' \cos^2\phi_2} \quad \ldots (18)$$

where ε_1 and ε_2 are the uncertainties in the measurement of J_b and J_a respectively. The inherent mean square error in K and subsequently in ΔH and ΔS can be evaluated as shown in the Appendix 1.

The frequency measurements are made from spectra recorded at 100 Hz sweep width, where one small division corresponds to 0.2 Hz. It could be readily seen from the spectra that the worst uncertainty is ±0.1 Hz. In most cases, the measurements are more precise. In addition, there are eight frequencies measured and only five parameters extracted from these; this amounts to an overdetermination of data points which abates error. Especially in the case of amino acids, each spectrum is recorded twice. Hence an uncertainty of ±0.06 Hz would be in order, representing an error of 11 cal/mol in ΔH and 0.036 cal/deg/mol in ΔS.

(*ii*) *Third conformer*

The free energy difference at 25° between rotamers II and III is approximated to be 1500 cal/mol. This estimate has very little bearing on the calculated ΔH^0 for the equilibrium between rotamers I and II; variation of the estimate from 1100 to 1900 cal/mol results in a change of ΔH by only 3 cal/mol.

(*iii*) *Ratio of J_t to J_g*

Table 2 shows that the ratio of Karplus constants and the dihedral angles are interdependent in the model. If

$$J_t = J_0\cos^2\phi_1 \quad \ldots (19)$$

and

$$J_g = J_0'\cos^2\phi_2 \quad \ldots (20)$$

it can be seen that the calculated thermodynamic parameters depend on the ratio, J_t/J_g. A value of 6.4 has been assigned to this ratio. This is equivalent to employing the original Karplus equation with $\phi_1 = 177°$ and $\phi_2 = 63°$. A deviation of 3° from perfect dihedral angles can be expected in 1,1,2-trisubstituted ethanes.

Table 2—Interdependence of Ratio of Karplus Constants and Dihedral Angles in the Model, 1-Iodo-2-methyl-3-phenylpropane

J_0/J_0'	ϕ_1°	ϕ_2°	J_t/J_g	ΔH (cal/mol)
1.600	180	60	6.4	−178
1.505	179	61	6.4	−178
1.412	178	62	6.4	−178
1.323	177	63	6.4	−178
1.236	176	64	6.4	−178
1.152	175	65	6.4	−178
1.070	174	66	6.4	−178

Results and Discussion

Intramolecular interactions between hydroxyl group and π-electrons has been the subject of several IR investigations[21]. Oki and Iwamura have examined $\nu O-H$ mode and found intramolecular interaction between the hydroxyl group and π-electrons in allyl alcohol and in 3-buten-1-ol[22]. However, no such bonding was detected in the higher homologs. They observed a similar trend in going from 2-arylethanols to the higher members of the series[23].

Snyder made a complete conformational analysis of 3-phenylpropanol and concluded that it possessed no intramolecular hydrogen bonding[24]. Detection of very weak intramolecular interaction in alcohols of the structure p-$R-C_6H_4-CH_2-CHMe-CH_2-OH$ (where $R=NO_2$, H, MeO) is reported here.

Concentration studies

The results of concentration studies on 2-methyl-3-phenyl-1-propanol are presented in Table 3; similar results are obtained with 3-(4-methoxyphenyl)-2-methyl-1-propanol and 2-methyl-3-(4-nitrophenyl))propan-1-ol. In all these cases, conformational free energy differences decrease with dilution. This trend is taken to indicate that associative interaction is more pronounced in the *trans* than in the *gauche* form.

The conformational energy difference in each case is extrapolated to infinite dilution to yield values of 306 cal/mol for the p-nitro alcohol, 200 for the unsubstituted one and 189 for the p-methoxy compound.

The *trans*-rotamer is the low energy form in all three alcohols, but the *gauche* form is stabilized by an additional 106 cal/mol in the unsubstituted alcohol and by 117 cal/mol in the p-methoxy one. This stabilization is attributed as due to intramolecular interaction.

$$\frac{\Delta G(NO_2)-\Delta G(MeO)}{\Delta G(NO_2)-\Delta G(H)}=\frac{117}{106}=1.10$$

Solvent effects

The conformational energy differences in aromatic solvents (Table 4) suggest that the hydroxyl group of the solute interacts more strongly with the heteroatoms than with the aromatic nuclei. Also, it may be pointed out that the aromatic solvents do not, to any extent, magnify the non-equivalence of the benzylic hydrogens.

The alcoholic solvents, as should be expected, are more strongly involved in H-bond formation with the solute. However, the smaller the solvent molecule, greater is its interaction with the gauche rotamer; hence a net reverse trend is manifested.

Neither the chemical shifts between the benzylic protons nor the conformational energies parallel solvent dielectric constants.

Variable temperature studies

The results for 2-methyl-3-(4-nitrophenyl)propan-1-ol are presented in Table 5; similar results are obtained for 2-methyl-3-phenyl-1-propanol and 3-(4-methoxyphenyl)-2-methyl-1-propanol. As the enthalpy values, show, the *trans* rotamer is the most stable one in all three alcohols (Table 6). However, the *gauche* conformer exhibits an additional stabilization of 88 cal in $C_6H_5-CH_2-CHMe-CH_2OH$ and of 92 cal in the p-methoxy alcohol, compared to their p-nitro analog. This stabilization must be the outcome of intramolecular interaction.

$$\frac{\Delta H(NO_2)-\Delta H(MeO)}{\Delta H(NO_2)-\Delta H(H)}=\frac{92}{88}=1.05$$

Correlation with theory

Four distinct models for intramolecular interaction exist in the literature, being proposed to ac-

Table 3—Changes in Spectral Parameters of $C_6H_5-CH_2-CHMe-CH_2OH$, with Change in Concentration in Chloroform Solution

Conc (w/w, %)	$\Delta\nu$ Hz*	J_a (Hz)	J_b (Hz)	J_+ (Hz)	J_{gem} (Hz)	ΔG (cal/mol)
100	48.815	8.712	5.460	14.172	−13.512	380
50	43.840	8.559	5.888	14.447	−13.497	308
25	40.344	8.526	5.985	14.511	−13.496	293
10	36.471	8.350	6.161	14.511	−13.531	251
5	33.883	8.338	6.254	14.592	−13.662	239
2	32.830	8.333	6.361	14.694	−13.639	226
1	32.672	8.065	6.228	14.293	−13.433	209

*At 100 MHz.

Table 4—Dependence of Spectral Parameters of $C_6H_5-CH_2-CHMe-CH_2OH$ on Solvent, at 0.33*M*

Solvent	$\Delta\nu$ Hz*	J_a (Hz)	J_b (Hz)	J_+ (Hz)	J_{gem} (Hz)	ΔG (cal/mol)
Hexane	43.109	8.151	6.117	14.286	−13.618	233
Benzene	42.386	8.369	6.704	15.073	−14.008	182
Benzene-F_6	37.364	8.296	6.267	14.563	−13.586	230
Anisole	44.118	8.573	6.287	14.860	−13.853	255
Nitrobenzene	44.270	8.412	5.889	14.301	−13.338	294
Aniline	43.590	8.597	5.852	14.449	−13.539	318
Methanol	44.601	8.624	5.957	14.581	−13.682	305
Ethanol	48.971	8.714	5.926	14.640	−13.695	318
t-Butanol	56.131	8.924	5.688	14.612	−13.793	374
Formic acid	24.862	8.058	6.297	14.355	−13.542	202

*At 100 MHz

Table 5a—Temperature Dependence of Spectral Parameters for p-$NO_2-C_6H_4-CH_2-CHMe-CH_2OH$, Solution (0.33*M*) in Chloroform

Temp (°C)	$\Delta\nu$ Hz*	J_a (Hz)	J_b (Hz)	J_+ (Hz)	J_{gem} (Hz)
105.2	35.749	8.007	5.982	13.989	−13.519
83.2	37.081	8.150	5.965	14.115	−13.499
61.4	38.623	8.343	5.913	14.256	−13.458
39.0	40.323	8.435	5.818	14.253	−13.424
23.3	32.373	8.567	5.788	14.355	−13.318
−1.2	44.919	8.696	5.690	14.386	−13.232
−23.9	47.747	8.919	5.580	14.499	−13.184
−46.7	50.640	9.207	5.295	14.502	−13.015

*At 100 MHz.

Table 5b—Conformational Analysis about C2-C3 Bond of p-$NO_2-C_6H_4-CH_2-CHMe-CH_2OH$

Temp (K)	n_1	n_2	n_3	ΔG^* (cal/nol)	ΔH^* (cal/mol)
378.2	0.564	0.374	0.062	308	396
356.2	0.577	0.373	0.050	309	393
334.4	0.595	0.368	0.037	320	398
312.0	0.604	0.359	0.037	322	395
296.3	0.616	0.356	0.028	323	392
271.8	0.628	0.347	0.025	321	384
249.1	0.649	0.337	0.014	325	383
226.3	0.676	0.310	0.014	350	403

*$\Delta H^0 = 393 \pm 18$ cal/mol; $\Delta S^0 = 0.234 \pm$ cal/deg/mol

Table 6—Energy Difference between Conformers I and II in $R-C_6H_4-CH_2-CHMe-CH_2-OH$

R	ΔH (Cal/mol)	ΔS (Cal/deg/mol)	$\Delta G_{25°}$ (Cal/mol)
NO_2	393	0.234	323
H	305	0.198	246
OMe	301	0.256	225

commodate infrared data: (i) in which benzene nucleus as a whole interacts[25]; (ii) a model in which the interaction involves only C-1 of the benzene ring[26]; (iii) a structure in which C-2 of the nucleus is the participant[27]; and (iv) one in which the π-bond between C-1 and C-2 takes part[28].

However, since MO theory excludes an orbital which would couple with that of a hydrogen atom,

the benzene nucleus as a whole is not expected to interact with the hydroxyl proton[29,30]. Since the observed interaction energies are similar for the unsubstituted and the methoxy substituted alcohols, Mulliken's predictions[29,30] are borne out by experiment.

Thus C-1 and/or C-2 of the benzene ring should be the site(s) of interaction. If the interaction were with C-1, Hammett's σ values would suggest a ratio of

$$\frac{\sigma_p(NO_2) - \sigma_p(MeO)}{\sigma_p(NO_2) - \sigma_p(H)} = \frac{1.05}{0.78} = 1.35 \qquad \ldots (21)$$

for the interaction energies of these alcohols. If only C-2 were to take part in the interaction, the ratio should be

$$\frac{\sigma_m(NO_2) - \sigma_m(MeO)}{\sigma_m(NO_2) - \sigma_m(H)} = \frac{0.59}{0.71} = 0.83 \qquad \ldots (22)$$

The expected value would be 1.09, if both C-1 and C-2 were equal partners. The experimental values of 1.10 and 1.05 are in agreement with this; hence the interaction is postulated to be with the '*ortho* double bond'.

Significance of entropy difference

Mizushima *et al.*[31] have suggested that the entropy difference between rotational isomers arises mainly from the difference in the internal rotation about the single bond. Mizushima *et al.*[32] have also observed that the frequencies of the torsional oscillations will become higher, due to internal hydrogen bonding; this will make the entropy of the bonded rotamer lower than that of the non-bonded one.

The *trans* form in the three alcohols is lower in entropy by 0.2 cal/deg/mol, compared to the *gauche* form (Table 6). We have seen earlier that there exist considerable solute-solute and solute-solvent interactions in these alcohols, and that these interactions are stronger in the *trans* than in the *gauche*. Presumably, these interactions involve the hydroxyl group, causing restrictions for the rotation about the C−O bond and lowering the entropy of the species. The second factor, namely the higher frequency of torsional oscillation caused by internal attraction, might be a minor contributor[23]. The difference in entropy are too small to warrant any further comment.

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APPENDIX 1

ERROR ANALYSIS

K, the equilibrium constant, is given by the expression (i)

$$K = \frac{n_2}{n_1} = \frac{J_b \pm \varepsilon_1 - J_0' \cos^2 \phi_2}{J_a \pm \varepsilon_2 - J_0' \cos^2 \phi_2} \qquad \ldots (i)$$

where ε_1 and ε_2 are the uncertainties in the measurement of the observed coupling constants J_b and J_a respectively.

The inherent error in K is then given by the expression (ii)

$$\delta K = \{(\partial K/\partial J_b)^2 \varepsilon_1^2 + (\partial K/\partial J_a)^2 \varepsilon_2^2\}^{1/2} \qquad \ldots (ii)$$

Substituting the differentials of Eq. (i),

$$\frac{\partial K}{\partial J_b} = \frac{1}{J_a \pm \varepsilon_2 - J_0' \cos^2 \phi_2}$$

and

$$\frac{\partial K}{\partial J_a} = -\frac{J_b \pm \varepsilon_1 - J_0' \cos^2 \phi_2}{J_a \pm \varepsilon_2 - J_0' \cos^2 \phi_2} = \frac{-K}{J_a \pm \varepsilon_2 - J_0' \cos^2 \phi_2}$$

in Eq. (ii), one obtains

$$\delta K = \{(\varepsilon_1/J_a \pm \varepsilon_2 - J_0' \cos^2 \phi_2)^2 + (K\varepsilon_2/J_a \pm \varepsilon_2 - J_0' \cos^2 \phi_2)^2\}^{1/2} \qquad \ldots (iii)$$

This can be rearranged to the form

$$\frac{\partial K}{K} = \varepsilon_1\{(1/J_b - J_0' \cos^2 \phi_2)^2 + (1/J_a - J_0' \cos^2 \phi)^2\}^{1/2} \qquad \ldots (iv)$$

when $\varepsilon_1 = \varepsilon_2$.

Since $\Delta G = -RT \ln K$,

$$\delta \Delta G = -\frac{RT}{K} \delta K \qquad \ldots (v)$$

Hence when the uncertainty in the measurement

of the coupling constant is 0.06 Hz, the inherent error in ΔG will be 11 cal/mol at 27°.

Similarly, since $\Delta H = -RT \ln K + T\Delta S$,

$$\delta\Delta H = -\frac{RT}{K}\,\delta K \qquad \ldots (vi)$$

Also, since $\Delta S = R \ln K + \frac{\Delta H}{T}$,

$$\delta\Delta S = \frac{R}{K}\,\delta K \qquad \ldots (vii)$$

Hence the inherent error in ΔS is 0.036 cal/deg/mol.

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Antiparasitic Agents: Part VI† — Synthesis of 1,2-,1,3- & 1,4-Bis(4-substituted aryloxy)benzenes & Their Biological Activities‡

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Several 1,2-, 1,3-, 1,4-bis(4-amidinophenoxy)benzenes (**8-10**); 1,2-, 1,3-, 1,4-bis(4-amidinobenzyloxy)benzenes (**14-16**) and 1-2-, 1,3-, 1,4-bis(4-amidinophenoxymethyl)benzenes (**26-28**) and the corresponding amidoximes (**17-19**), (**29-31**) and **33** have been synthesized. The compounds **8-10, 14-16** and **26-28** when tested *in vitro* against the promastigotes of *Leishmania donovani* have been found inactive at 50, 50 and 80 mg/ml concentrations. However, the compound **27** when tested *in vivo* against the amastigotes of *L. donovani* at a dose of 2.5 mg/kg × 5 days shows 56% inhibition and compound **16** 48% inhibition at 0.5 mg/kg × 5 days. The compounds **8-10** show 56, 39 and 69% inhibition *in vivo* at 2.5, 2.5 and 5 mg/kg × 5 days respectively. All the compounds have also been tested for their antimicrobial activity against several bacterial and fungal strains and some of these exhibit good antibacterial and antifungal activities.

Pentamidine (**1**) continues to be a useful drug for treating antimony-resistant leishmaniasis, despite its side effects, the most serious of which is the disturbance in sugar metabolism. The search, therefore, continues for newer antileishmanial agents as alternative to **1**.

It has been reported that the distance between the two intervening amidino functions in the antiprotozoal aromatic diamidines is crucial for determining their antiprotozoal activity[1]. This report prompted us to undertake the synthesis of pentamidine analogues in which the intervening methylene chain of 5 carbon atoms in pentamidine (**1**) is replaced by phenoxy, phenoxymethyl or benzyloxy moiety. In the present paper we report the synthesis of 1,2-, 1,3-, 1,4-bis(4-amidinophenoxy)benzenes (**8-10**); 1,2-, 1,3-, 1,4-bis(4-amidinobenzyloxy)benzenes (**14-16**) and 1,2-, 1,3-, 1,4-bis(4-amidinophenoxymethyl)benzenes (**16-18**) and **33** and the corresponding amidoximes **17-19** and **29-31** and their antileishmanial, antibacterial and antifungal activities.

Condensation of **2** (Scheme 1), **3**, and **4** separately with 4-chlorobenzonitrile gave the corresponding bis(4-cyanophenoxy)benzenes (**5-7**). Pinner reaction[2] of these compounds furnished the corresponding diamindines (**8-10**) in good yields.

Reaction of **2-4** (Scheme 2) separately with *p*-cyanobenzyl bromide gave the bis(4-cyanobenzyloxy)benzenes **11, 12** and **13**, respectively. Pinner reaction of these cyano compounds (**11-13**) afforded the corresponding diamindines (**14-16**). Treatment of **11-13** with hydroxyamine hydrochloride in aq. pyridine furnished the corresponding diaminodoximes (**17-19**).

Condensation of the isomeric α,α′-dibromoxylenes[3] (**20-22**) (Scheme 3) separately with 4-cyanophenol under basic condition gave the corresponding bis(4-cyanophenoxymethyl)benzenes (**23-25**) in good yields. Pinner reaction[2] of **23-25** afforded the diamindines **26-28**, respectively. Treatment of the cyano compounds (**23-25**) separately with hydroxylamine hydrochloride in aq. pyridine gave the corresponding diamidoximes (**29-31**).

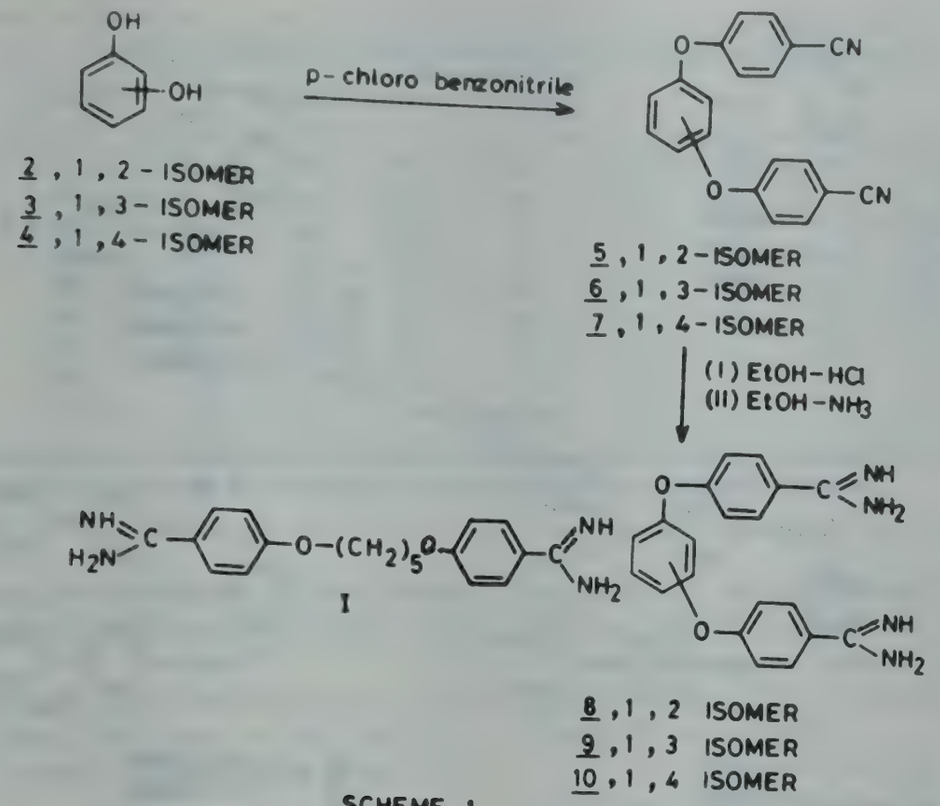
Condensation of 4-cyanophenol (Scheme 4) with methylene iodide yielded bis(4-cyanophenoxy)methane (**32**)[4]. Pinner reaction of **32** gave the diamindine (**33**).

Biological Activities

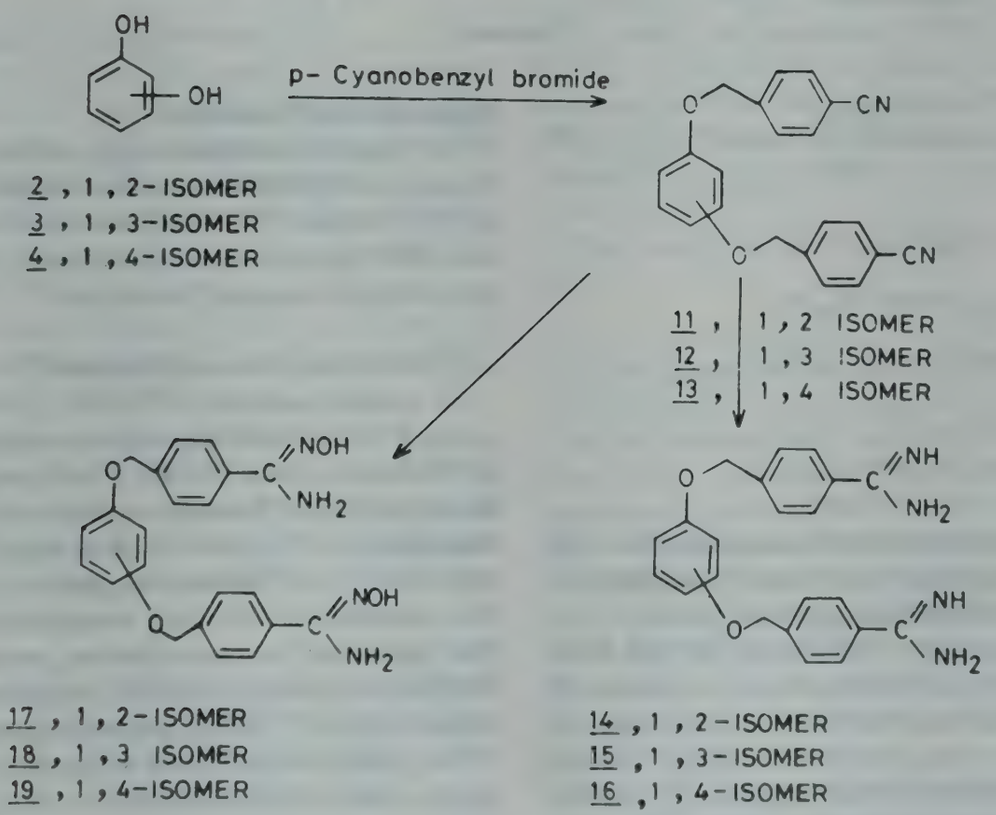
Antileishmanial activity (in vitro)

Collier and Lourie[5] medium was used to test the antileishmanial activity *in vitro* against the promastigotes of *L. donovani*. To the tubes containing sterile drug, 0.5 ml of medium and 0.1 ml of culture containing 0.5 million promastigotes, harvested from NNN medium, were added. The tubes were incubated at 25 ± 1°C and the end point (100% minimum inhibitory concentration in mg/ml) was recorded by

†For part V of this series, see ref. 6
‡CDRI Communication No. 4031
§Present address: Director, Rajendra Memorial Research Institute of Medical Sciences, Agamkuan, Patna (Bihar).


p- chloro benzonitrile
2 , 1 , 2 - ISOMER
3 , 1 , 3 - ISOMER
4 , 1 , 4 - ISOMER
5 , 1 , 2 - ISOMER
6 , 1 , 3 - ISOMER
7 , 1 , 4 - ISOMER
(I) EtOH - HCl
(II) EtOH - NH3
I
8 , 1 , 2 ISOMER
9 , 1 , 3 ISOMER
10 , 1 , 4 ISOMER

SCHEME 1

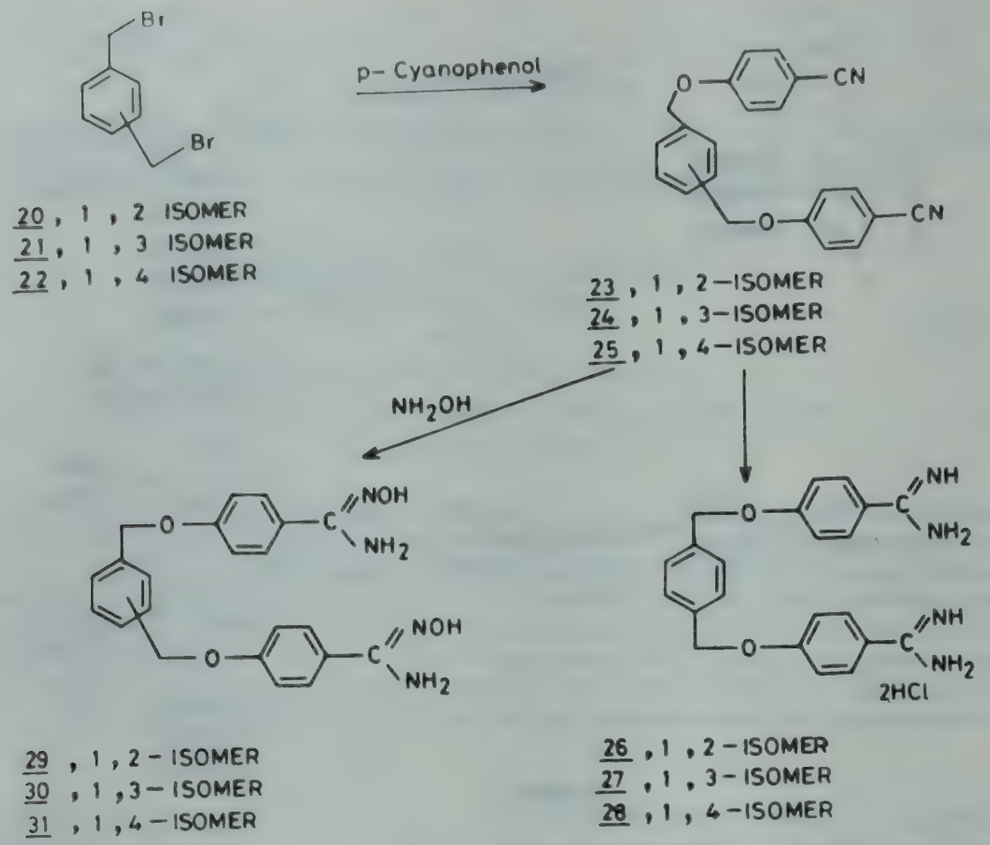

p- Cyanobenzyl bromide
2 , 1 , 2 - ISOMER
3 , 1 , 3 - ISOMER
4 , 1 , 4 - ISOMER
11 , 1 , 2 ISOMER
12 , 1 , 3 ISOMER
13 , 1 , 4 ISOMER
17 , 1 , 2 - ISOMER
18 , 1 , 3 ISOMER
19 , 1 , 4 - ISOMER
14 , 1 , 2 - ISOMER
15 , 1 , 3 - ISOMER
16 , 1 , 4 - ISOMER

Scheme 2

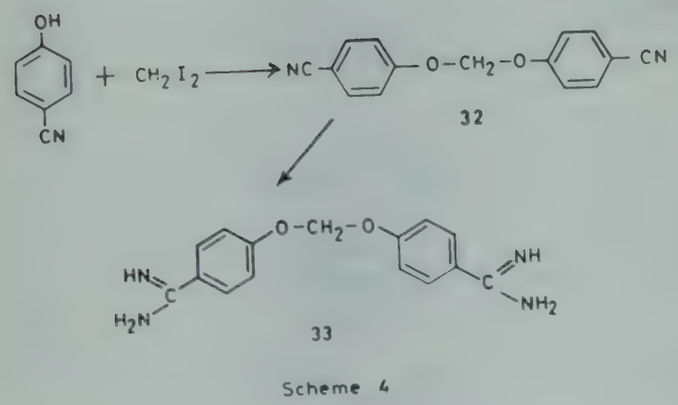
microscopic examination and by subculture in NNN medium.

Antileishmanial activity (in vivo)

Hamsters infected with *L. donovani* (Dd8 strain, Bihar) were used for testing the compounds. Infected hamsters with a count of 5 to 20 amastigotes per 100 spleen cell-nuclei were treated with one daily intraperitoneal injection of an ethanolic suspension (containing 1 to 2 drops of tween 80) of the compound for 5 days. The degree of infection (number of amastigotes per 100 spleen cell-nuclei) before and



Scheme 3



Scheme 4

after treatment in each animal was compared with that of the control group of animals. Pentamidine (**1**) dihydrochloride was used as positive control.

The compounds **8-10, 14-16** and **26-28** when tested *in vitro* were found inactive at 50, 50 and 80 mg/ml concentrations. However, compound **27** when tested *in vivo* at a dose of 2.5 mg/kg × 5 days exhibited 56% inhibition and compound **16** showed 48% inhibition at 0.5 mg/kg × 5 days. The compounds **8-10** showed 56, 39 and 69% inhibition *in vivo* at 2.5, 2.5 and 5 mg/kg × 5 days, respectively.

Antimicrobial activity

The compounds **8-10, 14-16, 26-28** and **33** were tested for their antimicrobial activity against several bacterial and fungal strains. The compounds which exhibited good activities are given in Table 1.

Experimental Procedure

For general directions, see an earlier paper[7]. C and H analyses of all the compounds reported in this paper were within ± 0.4% of theoretical values. The IR and PMR spectra of the compounds were in agreement with the assigned structures.

1,2-*Bis*(4-*cyanophenoxy*)*benzene* (**5**)

To a stirred solution of NaOEt (10 ml) (Na, 0.09 g in abs. EtOH, 10 ml) was added a solution of **2** (0.55 g, 0.005 mol, in abs. EtOH, 25 ml) and stirring continued for 1 hr. Thereafter a solution of 4-chlorobenzonitrile (1.50 g, 0.01 mol) in abs. EtOH (10 ml) was added to it. The mixture under N_2 atmosphere was refluxed for 2 hr and concentrated. The solid that separated out was filtered, washed with 2*N* NaOH, pet. ether and finally crystallized from ethyl acetate to give **5** (0.68 g, yield 43%), m.p. 162°.

The compounds **6** (yield 47%; m.p. 95-97°) and **7** were similarly prepared from **3** and **4**, respectively.

1,2-*Bis*(4-*amidinophenoxy*)*benzene* (**8**)

A mixture of **5** (1 g, 0.003 mol), dioxane (75 ml) and abs. EtOH (150 ml) (saturated with dry HCl gas at 0°) was stirred for 3 hr. The solvent and excess of HCl from the resulting mixture were removed under reduced pressure. Ethanolic ammonia (100 ml) (EtOH saturated with NH_3) was then added to it. The mixture was refluxed for 5 hr, the solvent and

Table 1—Antimicrobial Activity (MIC in μg/ml) of the Compounds

Compound	Bacteria*			Fungi*				
	A_1	A_2	A_3	B_1	B_2	B_3	B_4	B_5
8	In	In	12.5	In	25	25	In	In
10	In	In	12.5	In	In	In	In	In
18	25	25	In	In	In	In	25	In
19	12.5	25	25	In	In	In	In	25
26	25	25	In	In	In	25	25	0.39
27	12.5	25	12.5	25	25	25	12.5	1.54
28	12.5	25	12.5	25	In	25	25	0.39

In = Inactive

*A_1 = *Streptococcus faecalis*; A_2 = *Klebsiella pneumoniae*; A_3 = *Staphylococcus aureus*; B_1 = *Candida albicans*; B_2 = *Cryptococcus neoformans*; B_3 = *Sporotrichum schenckii*; B_4 = *Tricophyton mentagrophytes*; B_5 = *Aspergillus fumigatus*.

excess of ammonia were removed and the product was crystallized from 5*N*HCl to give **8**.2HCl (1.33 g, hyield 93%).

The compounds **9**. 2HCl (yield 80%; m.p. 125°) and **10**.2HCl [yield 70%; m.p. >300° (lit.[4] m.p. >300°)] were similarly prepared from **6** and **7** respectively.

1,2-*Bis*(4-*cyanobenzyloxy*)*benzene* (**11**)

A mixture of **2** (1.1 g, 0.01 mol), 4-cyanobenzyl bromide (3.92 g, 0.02 mol) and K_2CO_3 (3.52 g, 0.04 mol) in dry acetone (100 ml) was refluxed for 8 hr under N_2 atmosphere. The resulting mixture was filtered and the filtrate concentrated. The product, thus obtained, was crystallized from ethanol to give **11** (2.4 g, yield 70%), m.p. 120°.

The compound **12** (yield, 53%; m.p. 115°) and **13** were similarly prepared from **3** and **4**, respectively.

1,2-*Bis*(4-*amidinobenzyloxy*)*benzene* (**14**)

To an ice cooled solution of **11** (1.02 g, 0.003 mol) in abs. EtOH (250 ml) was passed dry HCl gas 1 hr and the reaction mixture kept at 0° for 6 hr and then stirred at ambient temperature for 4 hr. The solvent and excess of HCl from the resulting mixture were removed under reduced pressure. To it was added ethanolic ammonia (10 ml) (EtOH saturated with NH_3) and the mixture refluxed for 5 hr. The product, thus obtained, was crystallized from 5 *N*HCl to give **14**.2 HCl (0.8 g, yield 60%) m.p. 180°.

The compounds **15**.2 HCl (yield 46%; m.p. 135-37°) and **16** were similarly prepared from **12** and **13**, respectively.

1,2-*Bis*(4-*amidoximinobenzyloxy*)*benzene* (**17**)

A mixture of **11** (0.0025 mol), $NH_2OH.HCl$ (0.52 g, 0.001 mol), Na_2CO_3 (1.6 g, 0.01 mol), pyridine (15 ml) and EtOH (5 ml) was heated at 100° for 5 hr and filtered. The filtrate was concentrated and the solid, thus obtained, was crystallized from ethanol to give **17** (0.9 g, yield 90%), m.p. 180° (d).

The compounds **18** (yield 91%; m.p. 170°) and **19** (yield 84%; m.p. 300°) were similarly prepared from **12** and **13**, respectively.

1,2-*Bis*(4-*cyanophenoxymethyl*)*benzene* (**23**)

A mixture of α,α′-dibromo-*o*-xylene[3] (**20**) (2.64 g, 0.011 mol), *p*-cyanophenol (2.38 g, 0.02 mol) and K_2CO_3 (5.52 g, 0.04 mol) in dry DMF (20 ml) was refluxed for 6 hr. The resulting mixture was poured into H_2O (150 ml). The solid that separated out was crystallized from EtOH to give **23** (3 g, 90%), m.p. 154-55°.

The compound **25** was similarly prepared from **22**.

1,2-*Bis*(4-*amidinophenoxymethyl*)*benzene* (**26**)

A solution of **23** (1.7 g, 0.005 mol) in abs. EtOH (200 ml) was saturated with dry HCl gas at 0°. It was then stirred at ambient temperature for 3 hr. The solvent and excess of HCl from the resulting mixture were removed under reduced pressure. The base hydrochloride, thus formed, was refluxed with ethanolic ammonia (200 ml) (ethanol saturated with NH_3) for 4 hr, the solvent and excess of NH_3 were removed under reduced pressure and the product was crystallized from 5 *N*HCl to give **26**.2 HCl (1.84 g, yield 82%), m.p. 289-90°.

The compounds **27**.2 HCl (yield 86%; m.p. 209-11°) and **28**.2 HCl (yield 80%; m.p. 298°) were similarly prepared from **24** and **25**, respectively.

1,2-*Bis*(4-*amidoximinophenoxymethyl*)*benzene* (**29**)

A mixture of **23** (3, 4 g), pyridine (60 ml), EtOH (20 ml), H_2O (15 ml), $NH_2OH.HCl$ (3.15 g) and Na_2CO_3 (4.32 g in H_2O, 15 ml) was heated at 100° for 4 hr. The resulting mixture was diluted with H_2O

(50 ml). The solid that separated out was filtered and crystallized from pyridine-ethanol to give **29** (3.45 g, yield 85%), m.p. 234-35°.

The compounds **30** (yield 65%; m.p. 210°) and **31** (yield 50%; m.p. 205-07°) were similarly prepared from **24** and **25** respectively. The compound **33** was prepared from **32**[4].

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Synthesis & Pharmacological Evaluation of N^1-[*p*-(3-Aryl-4-oxoquinazolin-2-ylmethylamino)benzoyl]-N^4-arylthiosemicarbazides & 2-Arylamino-5-[*p*-(3-aryl-4-oxoquinazolin-2-ylmethylamino)phenyl]-1,3,4-thiadiazoles/oxadiazoles as Oral Hypoglycemic Agents

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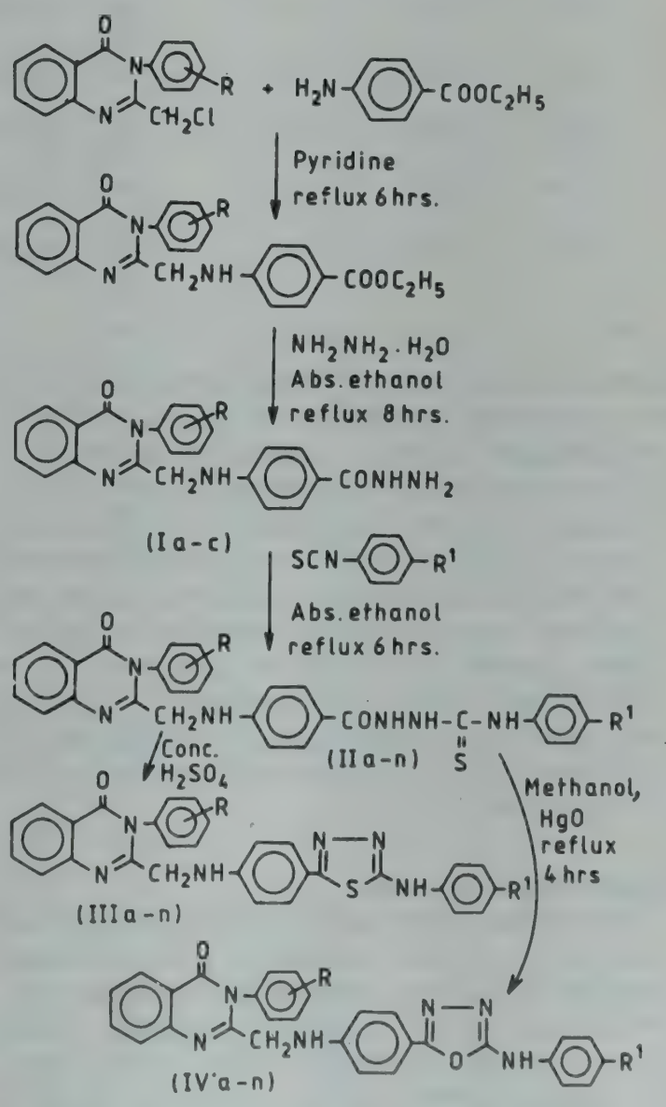
A series of 2-arylamino-5-[*p*-(3-aryl-4-oxoquinazolin-2-ylmethylamino)phenyl]-1,3,4-thiadiazoles/oxadiazoles (III/IV) have been synthesised from N^1-[*p*-(3-aryl-4-oxoquinazolin-2-ylmethylamino)benzoyl]-N^4-arylthiosemicarbazides (II) by treatment with conc. sulphuric acid or mercuric oxide. The thiosemicarbazides (II), in turn, hve been prepared by refluxing 2-[*p*-hydrazinocarbonyl)phenylaminomethyl]-3-arylquinazolin-4(3*H*)-one (I) with various aryl isothiocyanates in abs. ethanol. A few of these compounds when tested on rats at an oral dose of 250 mg/kg, reduced the blood sugar to about 25%.

Quinazolone derivatives have been found associated with antiinflammatory[1], anthelmintic[2], anticonvulsant[3] and CNS depressant[4] activities. Few quinazolone derivatives including 2-piperazino-4(3*H*)-quinazolone monoacetate[5] have also been recently shown to exhibit hypoglycemic activity. Moreover, it was observed that 5-alkyl-2-amino-1,3,4-thiadiazole derivatives[6], particularly glybutthiazol[7] (glipsol), N^5-aryl-1,3,4-oxadiazol-2-ylaminocarbonyl)-4-methylpiperazinobiguanides[8] as well as thiosemicarbazides[9] display a high order of hypoglycemic activity. Based on these findings, it was considered desirable to synthesise the title compounds with the hope that the incorporation of thiadiazole or oxadiazole moiety in substituted quinazolones might enhance their hypoglycemic activity. The desired thiadiazoles and oxadiazoles were synthesised as shown in Scheme 1.



Scheme 1

Hypoglycemic activity

A group of albino rats were kept on fast for 18 hr and water was allowed *ad libitum* prior to experiment. The fasting blood sugar samples were drawn from the tail veins of the rats and their glucose content was determined by Somogyi method[10]. The compounds to be tested were emulsified with 5% gum tragacanth and administered orally at a single dose of 250 mg/kg body weight. The blood samples were again drawn from the tail veins at intervals of 1,2 and 4 hr and their glucose content was again determined. The percentage change of glucose level was calculated.

From the results recorded in Tables 1 and 2, it

Table 1—Characterization Data of N^1-[*p*-3-Aryl-4-oxoquinazolin-2-ylmethylamino)benzoyl]-N^4-arylthiosemicarbazides (II)

Compd	R	R^1	m.p. °C	Mol. formula	Found (%) (Calc.)			Hypoglycemic potency (% reduction in blood sugar)
					C	H	N	
IIa	*o*-CH_3	H	124	$C_{30}H_{26}N_6O_2S$	67.1 (67.4	4.7 4.9	15.8 15.7)	16
IIb	*p*—OCH_3	H	116	$C_{30}H_{26}N_6O_3S$	65.6 (65.5	4.7 4.7	15.1 15.3)	—
IIc	*p*-NO_2	H	142	$C_{29}H_{23}N_7O_4S$	61.8 (61.6	4.0 4.1	17.1 17.4)	8
IId	*o*-CH_3	CH_3	144	$C_{31}H_{28}N_6O_2S$	67.7 (67.9	5.2 5.1	15.0 15.3)	—
IIe	*p*-OCH_3	CH_3	130-2	$C_{31}H_{28}N_6O_3S$	66.2 (66.0	4.8 5.0	14.8 14.9)	17
IIf	*p*-NO_2	CH_3	162	$C_{30}H_{25}N_7O_4S$	62.0 (62.2	4.0 4.3	16.7 16.9)	—
IIg	*o*-CH_3	OCH_3	152	$C_{31}H_{28}N_6O_3S$	65.8 (66.0	4.9 5.0	14.7 14.9)	14
IIh	*p*-OCH_3	OCH_3	122	$C_{31}H_{28}N_6O_4S$	64.0 (64.1	4.6 4.8	14.8 14.5)	—
IIi	*p*-NO_2	OCH_3	202	$C_{30}H_{25}N_7O_5S$	60.3 (66.5	4.4 4.2	16.6 16.5)	—
IIj	*o*-CH_3	Cl	180	$C_{30}H_{25}N_6O_2SCl$	63.8 (63.7	4.3 4.4	14.7 14.9)	13
IIk	*p*-OCH_3	Cl	140	$C_{30}H_{25}N_6O_3SCl$	61.4 (61.6	4.3 4.3	14.4 14.4)	—
IIl	*p*-NO_2	Cl	172	$C_{29}H_{22}N_7O_4SCl$	57.8 (58.0	3.8 3.7	16.5 16.4)	—
IIm	*o*-CH_3	Br	164	$C_{30}H_{25}N_6O_2SBr$	58.3 (58.7	4.0 4.1	13.5 13.7)	12
IIn	*p*-OCH_3	Br	192	$C_{30}H_{25}N_6O_3SBr$	57.0 (57.2	4.1 4.0	13.3 13.4	—

is difficult to arrive at a definite trend in SAR of the compounds synthesised. In the case of thiosemicarbazides (Table 1) maximum reduction of 17% was shown by compound IIe with R = *p*-OCH_3 and $R^1 = CH_3$. The results recorded in Table 2 reveal that incorporation of a thiadiazole moiety in substituted quinazolones results in a greater hypoglycemic activity than incorporation of an oxadiazole moiety. However, the presence of *p*-OCH_3(R) and CH_3(R^1) in compounds of both the series causes maximum reduction in blood glucose as shown by the hypoglycemic activity of IIIe (25%) and IVe (18%).

Experimental Procedure

All melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded in KBr on a Perkin-Elmer spectrophotometer (ν_{max} in cm^{-1}) and PMR spectra in $CDCl_3$ on a Varian A60D instrument using TMS as internal standard (chemical shifts in δ, ppm). Purity of the compounds was checked by TLC using plates coated with silica gel G.

2-[(p-Hydrazinocarbonyl)phenylaminomethyl]-3-(o-tolyl)quinazolin-4(3H)-one (Ia)

A mixture of 2-chloromethyl-3-(*o*-tolyl)quinazolin-4-(3*H*)-one[11] (11.4 g, 0.04 mol) and ethyl *p*-aminobenzoate (6.6 g, 0.04 mol) in pyridine (20 ml) was refluxed for 6 hr, pyridine distilled off and the residue poured onto crushed ice containing a few drops of hydrochloric acid with stirring. The solid thus separated was filtered, washed with a little cold water, and refluxed with hydrazine hydrate (3.9 ml, 0.08 mol) in abs. ethanol (25 ml) for 12 hr. On cooling the reaction mixture, a solid separated out which was filtered and recrystallised from ethanol to give Ia, yield 65%, m.p. 152° (Found: C, 69.1; H, 5.0; N, 17.7. $C_{23}H_{21}N_5O_2$ requires C, 69.2; H, 5.3; N, 17.5%); IR: 3400-3320 (NH and NH_2), 1680 (C = O) and 1620 (C = N).

Similarly, other compounds of the type I were prepared in 60-62% yields. Their characterization data are as follows:

Ib (R = *p*-OCH_3): m.p. 146° (Found: C, 66.6; H,

Table 2—Characterisation Data of 2-Arylamino-5-[*p*-(3-aryl-4-oxoquinazolin-2-ylmethylamino)phenyl]-1,3,4-thiadiazoles (III/IV)

Compd	R	R^1	m.p. °C	Mol. formula	Found (%) (Calc.)			Hypoglycemic potency (%) reduction in blood sugar
					C	H	N	
IIIa	*o*-CH_3	H	140	$C_{30}H_{24}N_6OS$	69.6 (69.8	4.8 4.7	16.0 16.3)	17
IIIb	*p*-OCH_3	H	146	$C_{30}H_{24}N_6O_2S$	67.8 (67.7	4.3 4.5	15.7 15.8)	—
IIIc	*p*-NO_2	H	166	$C_{29}H_{21}N_7O_3S$	63.3 (63.6	3.6 3.8	18.2 17.9)	—
IIId	*o*-CH_3	CH_3	174	$C_{31}H_{26}N_6OS$	70.4 (70.2	4.8 4.9	15.7 15.9)	—
IIIe	*p*-OCH_3	CH_3	156	$C_{31}H_{26}N_6O_2S$	64.4 (68.1	4.9 4.8	15.3 15.4)	25
IIIf	*p*-NO_2	CH_3	182	$C_{30}H_{23}N_7O_3S$	64.0 (64.2	4.0 4.1	17.8 17.5)	—
IIIg	*o*-CH_3	OCH_3	190-92	$C_{31}H_{26}N_6O_2S$	68.4 (68.1	4.6 4.8	15.2 15.4)	21
IIIh	*p*-OCH_3	OCH_3	188	$C_{31}H_{26}N_6O_3S$	66.0 (66.2	4.5 4.6	14.8 14.9)	—
IIIi	*p*-NO_2	OCH_3	224	$C_{30}H_{23}N_7O_4S$	62.0 (62.4	4.2 4.0	16.9 17.0)	13
IIIj	*o*-CH_3	Cl	208	$C_{30}H_{23}N_6OSCl$	(65.1 (65.4	4.4 4.2	15.5 15.3)	16
IIIk	*p*-OCH_3	Cl	198	$C_{30}H_{23}N_6O_2SCl$	63.8 (63.6	4.0 4.1	14.6 14.8)	—
IIIl	*p*-NO_2	Cl	204-06	$C_{29}H_{20}N_7O_3SCl$	(59.8 (59.9	3.2 3.4	17.1 16.9)	—
IIIm	*o*-CH_3	Br	212-14	$C_{30}H_{23}N_6OSBr$	60.2 (60.5	3.7 3.9	14.0 14.1)	14
IIIn	*p*-OCH_3	Br	232	$C_{30}H_{23}N_6O_2SBr$	58.7 (58.9	3.6 3.8	13.5 13.8)	—
IVa	*o*-CH_3	H	134	$C_{30}H_{24}N_6O_2$	72.2 (72.0	4.6 4.8	16.5 16.8)	15
IVb	*p*-OCH_3	H	138	$C_{30}H_{24}N_6O_3$	69.6 (69.8	4.8 4.7	16.0 16.3)	—
IVc	*p*-NO_2	H	142	$C_{29}H_{21}N_7O_4$	65.4 (65.5	3.9 4.0	18.8 18.5)	—
IVd	*o*-CH_3	CH_3	162-64	$C_{31}H_{26}N_6O_2$	72.2 (72.4	5.0 5.1	16.1 16.3)	—
IVe	*p*-OCH_3	CH_3	146	$C_{31}H_{26}N_6O_3$	70.4 (70.2	4.8 4.9	15.8 15.9)	18
IVf	*p*-NO_2	CH_3	152	$C_{30}H_{23}N_7O_4$	66.4 (66.1	4.0 4.2	18.2 18.0)	—
IVg	*o*-CH_3	OCH_3	180	$C_{31}H_{26}N_6O_3$	70.3 (70.2	4.8 4.9	15.7 15.9)	17
IVh	*p*-OCH_3	OCH_3	172	$C_{31}H_{26}N_6O_4$	68.0 (68.1	4.6 4.8	15.6 15.4)	—
IVi	*p*-NO_2	OCH_3	202	$C_{30}H_{23}N_7O_5$	64.4 (64.2	4.0 4.1	17.6 17.5)	—
IVj	*o*-CH_3	Cl	194	$C_{30}H_{23}N_6O_2Cl$	67.0 (67.4	4.1 4.3	15.8 15.7)	15
IVk	*p*-OCH_3	Cl	182	$C_{30}H_{23}N_6O_3Cl$	65.3 (65.4	4.4 4.2	15.2 15.3)	—
IVl	*p*-NO_2	Cl	190	$C_{29}H_{20}N_7O_4Cl$	61.1 (61.5	3.3 3.5	17.5 17.3)	—
IVm	*o*-CH_3	Br	196	$C_{30}H_{23}N_6O_2Br$	62.0 (62.2	4.2 4.0	14.2 14.5)	10
IVn	*p*-OCH_3	Br	214-16	$C_{30}H_{23}N_6O_3Br$	60.2 (60.5	3.7 3.9	14.0 14.1	—

5.2; N, 16.7. $C_{23}H_{21}N_5O_3$ requires C, 66.5; H, 5.1; N, 16.9%).

Ic (R = *p*-NO_2): m.p. 164° (Found: C, 61.2; H, 4.0; N, 19.2. $C_{22}H_{18}N_6O_4$ requires C, 61.4; H, 4.2; N, 19.5%).

N^1-[p-(3-o-Tolyl-4-oxoquinazolin-2-ylmethylamino)-benzoyl]-N^4-phenylthiosemicarbazide (IIa)

Compound Ia (12 g, 0.03 mol) was refluxed with phenyl isothiocyanate (4.1 ml, 0.03 mol) in abs. ethanol (30 ml) for 6 hr. On cooling a solid separated out which was recrystallised from ethanol to give IIa, yield 63%, m.p. 124° (Found: C, 67.1; H, 4.7, N, 15.8. $C_{30}H_{26}N_6O_2S$ requires C, 67.4; H, 4.9; N, 15.7%); IR: 3350 (−NH), 1685 (C=O), 1615 (C=N) and 1080, 1130 (C=S); PMR: 6.8-7.7 (*m*, 17H, Ar−*H*), 2.1 (*s*, 3H, Ar−C*H*$_3$), 3.8 (*s*, 2H, −CH_2−N) and 8.0-8.6 (*m*, 4H, CONHNHCSN*H* and CH_2N*H*).

Similarly, other compounds of the type II were prepared in 50-62% yields. Their characterization data are given in Table 1.

2-Phenylamino-5-[p-(3-o-tolyl-4-oxoquinazolin-2-ylmethylamino)phenyl]-1,3,4-thiadiazole (IIIa)

A solution of IIa (5.3 g, 0.01 mol) in conc. sulphuric acid (10 ml) was kept at room temperature for 4 hr while stirring at intervals. Thereafter, it was poured onto crushed ice, and the separated solid recrystallised from ethanol, yield 60%, m.p. 140° (Found: C, 69.6; H, 4.8; N, 16.0. $C_{30}H_{24}N_6OS$ requires C, 69.8; H, 4.7; N, 16.3%); IR: 3320 (−NH), 1680 (C=O) and 1620 (C=N); PMR: 6.9-7.8 (*m*, 17H, Ar−*H*), 2.2 (*s*, 3H, Ar−C*H*$_3$), 3.8 (*s*, 2H, −CH_2−N) and 8.1-8.2 (*s*, 2H, CH_2N*H* and Ar−N*H*).

Similarly, other compounds of the Type III were prepared in 55-60% yield. Their characterization data are given in Table 2.

2-Phenylamino-5-[p-(3-o-tolyl-4-oxoquinazolin-2-ylmethylamino)phenyl]-1,3,4-oxadiazole (IVa)

To a solution of IIa (5.3 g, 0.01 mol) in methanol (50 ml) was added mercuric oxide (2.6 g, 0.012 mol), and the reaction mixture refluxed for 4 hr, filtered, solvent distilled off and the residue recrystallised from ethanol to give IVa, yield 58%, m.p. 134° (Found: C, 72.2; H, 4.6; N, 16.5. $C_{30}H_{24}N_6O_2$ requires C, 72.0; H, 4.8; N, 16.8%); IR: 3325 (−NH), 1680 (C=O) and 1620 (C=N); PMR: 6.8-7.6 (*m*, 12H, Ar−*H*), 2.2 (*s*, 3H, Ar−C*H*$_3$), 3.8 (*s*, 2H, −CH_2−N) and 8.0-8.1 (*s*, 2H, CH_2N*H* and Ar−N*H*).

Similarly, other compounds of the type IV were prepared in 55-58% yields. Their characterization data are given in Table 2.

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Studies on Physico-chemical Properties of Biologically Important Benzodiazepines: Part I—Ionization & Carbinol Formation

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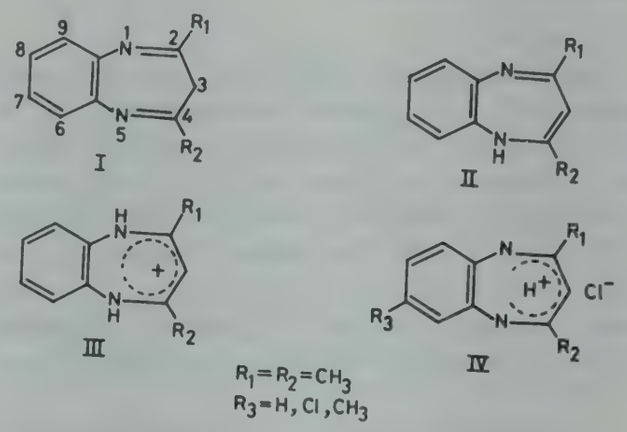
The structural characteristics of dimethylbenzo-1,5-diazepine cation in aq. medium has been reconsidered in the light of unusual *p*H-titration behaviour. The two-stage pK_a values observed may lead to several ionization schemes. The possibility of covalent hydration and/or carbinol formation has been discussed on the basis of *p*H, conductometric and spectrophotometric kinetic studies. The experimental evidences support the eventual introduction of a secondary alcoholic group in benzodiazepine molecule. This may open new vistas in understanding its potential drug activity.

Compounds having a condensed diazepine nucleus, especially the benzodiazepines[1-3], have attracted the attention of several chemists engaged in the synthesis of organic compounds and biologists because of their invaluable therapeutic properties. Among the simple benzodiazepines, 2,4-disubstituted 6,7-benzo-1,5-diazepine has been the key molecule in elucidating the structure of related compounds. Eversince Thiele and Steimmig[4] synthesised 2,4-dimethyl-6-7-benzo-1,5-diazepine (DMBDA), the structure seems to be eluding concrete assignments. Several resonating structures (I and II) have been proposed by a number of workers based on IR, electronic, PMR and X-ray studies[5-8]. Among them, the salient ones are those proposed by Lloyd and Marshall[5] and Vaisman[6]. Finar[7] has demonstrated the presence of a $-CH_2$ group in structure I while Turchin[9] has shown the presence of paramagnetic ring current in structure III by PMR and LCAO-MO calculations.

A few pK_a determinations have also been reported[10,11]. Yet, there appears to be some cloud about the structural behaviour of the base I, that too, in solution phase, thus needing a reinvestigation.

None of the earlier workers have considered the possibility of covalent hydration and carbinol formation in benzodiazepine molecule. Benzodiazepines are biologically active and have suitable sites for possible hydration. It has been predicted that many aspects of covalent hydration would be of paramount interest to biologists[12]. Most of the six-or higher-membered nitrogen heterocycles which are prone to covalent hydration are biologically active substances[13]. The distribution of substances between water and lipids has also been investigated taking into account the covalent hydration[13]. It has been reported that benzodiazepine drugs usually undergo a metabolic change before they act[14]. One of the metabolic steps involves hydroxylation yielding an active derivative.



In the light of the above observations we report in this paper the results of *p*H-metric, conductometric and spectrophotometric kinetic studies of the compounds of general formula IV.

Materials and Methods

DMBDA and its derivatives were prepared and recrystallised by literature methods[4].

Stock solution of DMBDA and solutions of various concentrations were prepared by standard procedure in carbon dioxide-free doubly distilled water. All titrations were carried out under nitrogen atmosphere at 25°.

Absorption spectra were recorded on an ELICO CL 24 spectrophotometer while *p*H and conductance were measured on a digital ELICO model LI 122 *p*H-meter and an ELICO type CM 82T conductivity bridge with a cell (Cell constant 0.982 cm^{-1}), respectively.

Kinetics by spectrophotometric method

Procedure similar to that of Isaacs[15] was adopted.

pH-titrations

*p*H-titrations were carried out by Cavagnol's method[16]. DMBDA and its derivatives were titrated against 1.5 *M* NaOH. For testing the formation of hysterisis loop, the titration was done with 0.1 *M* NaOH. The titrated solution was back titrated with 0.1 *M* HCl.

Conductometric titrations

DMBDA and its derivatives were titrated against 0.1 *M* NaOH solution. During titration a stream of nitrogen gas was passed through the contents.

Results and Discussion

pH-titrations

The *p*H-titrations of DMBDA's gave rise to two distinct inflection points corresponding to two pK_a's (Table 1) in the absence of any salt added. The *p*H curves are shown in Fig.1. Such distinct inflections for DMBDA have not been reported so far. Also, during the course of titration it was noticed that the *p*H takes some time (nearly 10 to 30 min) to remain stable between *p*H 4 and 7. This strange behaviour during *p*H titration may be due to some structural variations in DMBDA molecule.

Table 1—Ionization & Rate Constants for 7-Substituted 1,5-Dimethylbenzodiazepines (solvent, water; temperature, 25°)

Sl. No.	Compound	$\approx pk_{a_1}$	$\approx pK_{a_2}$	Rate constant* (in s^{-1}) $k = \frac{2.303}{t} \log \frac{A_0}{A_t}$
1	DMBDA	2.0	5.5	9.66×10^{-3}
2	7-Me-DMBDA	3.5	5.8	2.64×10^{-3}
3	7-Cl-DMBDA	3.8	5.0	4.02×10^{-3}

*From the plot of $\log A_0/A_t$ versus t

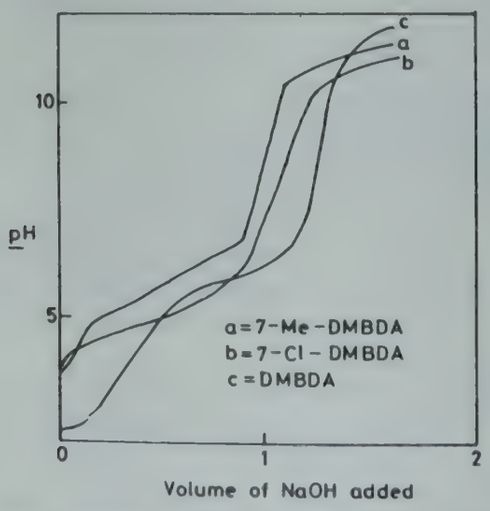
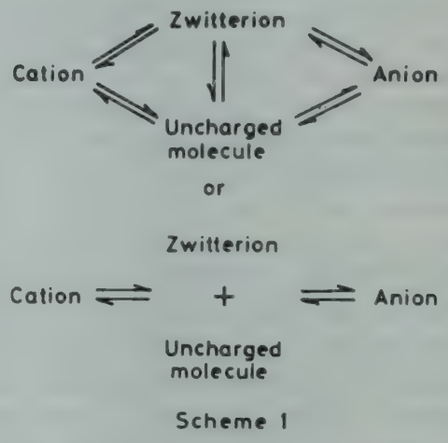


Fig. 1—*p*H Titration curves of DMBDA & its 7-substituted derivatives

The two-stage ionization observed for 1,5-benzodiazepine system can be discussed on the following lines.

Ijzerman *et al.*[17] have given an ionization scheme for dibasic β-adrenoceptor agonists as shown in Scheme 1.



Scheme 1

However, Barrett et al.[18] proposed the two-stage ionization mechanism as $H_2A^+ \rightleftarrows HA \rightleftarrows A^-$ for a few 1,4-benzodiazepine drugs on the basis of structural data. Further, Clifford and Smyth[19] predicted that 1,4-benzodiazepines possessing a second acid dissociation constant may exist as neutral molecules at *p*H greater than pK_1 and at *p*H less than pK_2. And as neutral molecules, these drugs are generally extracted from body fluids into non-aqueous solvents.

In the light of these reports, one can expect a similar ionization mechanism for the 1,5-benzodiazepine systems. However, for these mechanisms the second stage ionization may have a value around 10. The pK_2 value obtained by us in the present study is as low as ≈ 5.5. This may be due to ring opening and ring closing processes.

The observed drift in *p*H between 4 and 7 during titration may arise due to several factors. However, only two factors are given here.

(1) Decomposition of DMBDA should result in benzimidazole and ketone (Scheme 2). We took equimolar mixtures of benzimidazole and acetone, and titrated with NaOH conductometrically. This titration curve was not in agreement with the conductometric titration curve for DMBDA versus NaOH of similar concentration. This clearly shows that DMBDA does not decompose during titration interval and also with the concentration of NaOH used. This was further supported by reverse *p*H-titration.

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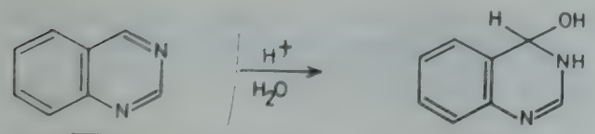
Scheme 2



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Scheme 3

(2) The drift in *p*H value during titration may also be due to covalent hydration[13]. Many aromatic N-heterocycles add water across the C-N double bond[13] as shown in Scheme 3.

Hydration increases as the number of ring nitrogen increases and the cations are more strongly hydrated than the neutral species. One essential feature for hydration is the presence of an electron-withdrawing centre which is capable of depleting the π-electron layer. Doubly bonded nitrogens that too in odd numbered positions viz. 1,3; 1,5; etc., enhance the electron-withdrawing force additively. Thus, the compound under consideration (DMBDA) seems to be tailor-made for covalent hydration.

While reporting C=N hydration in 6-hydroxypteridines Albert *et al.*[20] observed a strange behaviour during *p*H-titration. With alkali, a curve is traced corresponding to a weak acid. But on back titration with acid, this curve is not retraced. Instead, a new curve appears corresponding to much stronger acid. The hydrated species is a weaker acid than the anhydrous species and hence a hysterisis loop is obtained. We have observed a similar hysterisis loop as shown in Fig.2 during back titration of DMBDA, demonstrating possible covalent hydration. Furhter, Goldacre and Phillips[21] have attributed the drift in *p*H to the carbinol formation in the case of crystal violet. This is further supported by spectrophotometric kinetic studies[22].

Perrin[23] has reported that reversible hydration of nitrogen-containing heterocyclic bases is acid-base catalysed, and at constant *p*H the reactions obey first order rate expression.

Our similar kinetic results agreed well with this first order rate expression, as shown by the constant *k* values given in Table 1. The DMBDA solution got decolourised after keeping with NaOH. Thus, DMBDA may form a carbinol in aq. medium as shown in Scheme 4 and covalent hydration may be viewed as an intermediate step. This is also termed a 'bleaching' reaction.

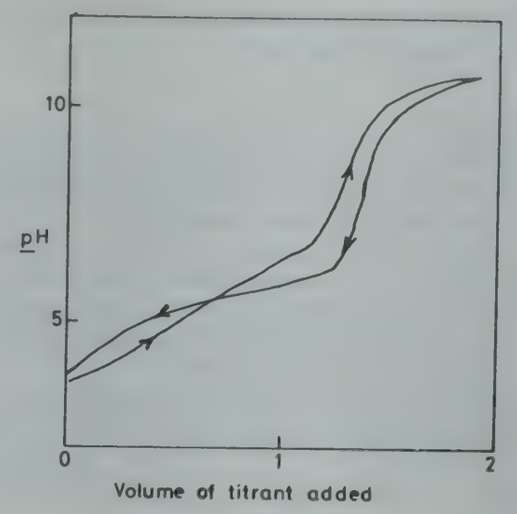
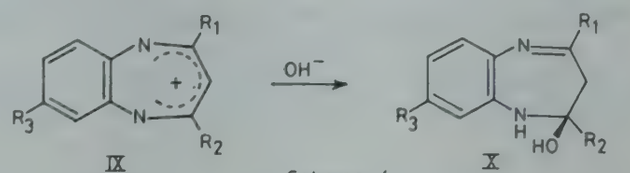


Fig. 2—Hysterisis loop formed during back titration of DMBDA with 0.1 *M* HCl



IX X

Scheme 4

Conductometric titrations

The two-stage ionization observed for DMBDA was further supported by conductometric titrations. The titration curve looked similar to that of a mixturse of strong and weak acids against a strong base. This may be indicative of the relative strengths of the two possible ionizing groups in the molecule.

Conclusion

The available experimental evidences with us at present, support the carbinol formation through covalent hydration of DMBDA molecule. The hydroxylation (carbinol formation) at either C-2 or C-4 results in a secondary alcoholic group. Ring opening if any, may also result in a hydroxylated molecule. This may be of paramount importance in correlating the probable participation of this group in the biological activity of benzodiazepine systems on track with the reported role of secondary alcoholic group, in enzyme action and biological activity as referred in introduction[13].

Acknowledgement

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Structural Studies on Polysaccharide from *Pseudomonas aeruginosa* Habs Type 1

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The O-specific polysaccharide (F-1 fraction) from the *Pseudomonas aeruginosa* Habs Type 1 has been isolated and purified by chromatography. Tentative structure (I) has been suggested for the repeating unit of the polysaccharide based on acid hydrolysis, methylation and periodate oxidation studies.

Pseudomonas aeruginosa is a gram negative bacteria causing a variety of Septic infections[1] in men and other vertebrates. It is a potentially dangerous, opportunistic pathogen. Although the human immunological sytem is normally effective in protecting the healthy host against this organism, severe and frequent lethal infections are common in patients whose immunological defence system has been weakened by long illness or by trauma arising from severe burns or from surgery. Infections resulting from virulent strains of *P. aeruginosa* are especially troublesome if they become established in hospitals. Patients having extensive burns are particularly prone to *Pseudomonas* sepsis and common antimicrobial agents are largely ineffective in combating it.

Of the 16 serotypes of *Pseudomonas aeruginosa* so far encountered, seven were found to be more prominent compared to the others. *P. aeruginosa* Habs Type 1 is one such serotype[2]. Some of the Fisher types[3] and Lanyi types[4] of *P. aeruginosa* were studied by other workers[5,6]. In continuation of our work in ascertaining the relationship between the structure of antigens and the immunological properties of the microbial cell, we started working on the *P. aeruginosa* Habs Type 1 O-specific polysaccharide. We now describe the structural elucidation of this O-specific polysaccharide.

Pseudomonas aeruginosa Habs Type 1 was cultured in nutrient broth[7] in roux bottles. The growths were harvested and dry cells were isolated. Lipopolysaccharide (LPS) was extracted from the dry cells with phenol-water[8]. The total water extract was dialysed and lyophilized. Nucleic acid was removed by precipitation with cetavlon and crude LPS was obtained by precipitation by the addition of ethanol. The crude LPS thus obtained was purified by passing through a column of Sephadex G-100 using pyridine - acetic acid - water (4:10:1000) as solvent. It came out as a broad peak. The LPS was delipidified with 1% acetic acid to give the polysaccharide (PS). The PS was charged onto a Sephadex G-100 column when two peaks were obtained (Fig.1). The first fraction (F-1) was found to give a sharp precipitin band with the homologous antiserum raised in rabbits. The second fraction (F-2) appearing beyond the void volume gave a faint broad band. The fraction F-2 was found to contain low molecular weight fragments.

Acid hydrolysis of F-1 fraction gave L-rhamnose, D-glucose and L-fucosamine in the ratio 2:1:1 (columns A and B) indicating the possibility of a tetrasaccharide repeating unit in the antigen. The F-2 fraction, on hydrolysis gave the same sugars in the ratio 1:2:1. It is possible that this frac-

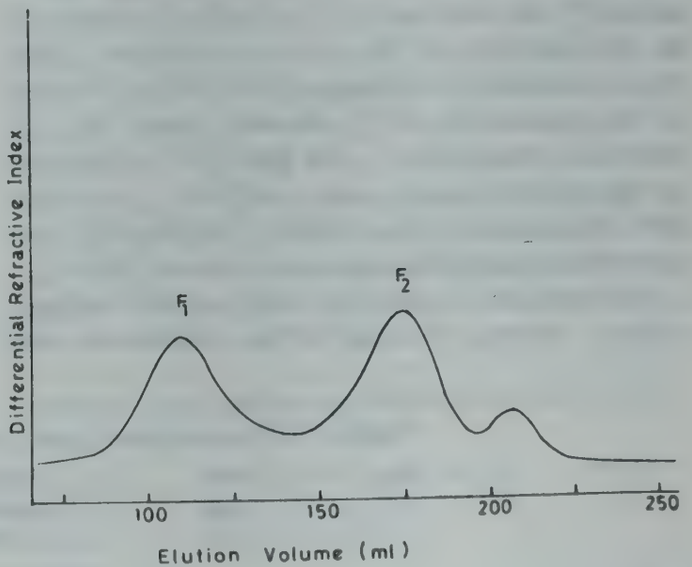


Fig. 1—Monitoring by differential refractometer of various fractions obtained on passing crude polysaccharide through a column of Sephadex G-100 using pyridine-acetic acid - water (4:10:1000) as eluent (80 fractions of 5 ml each were collected and monitored)

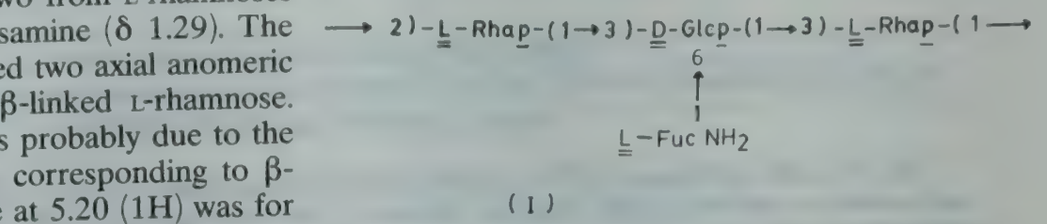
tion has appeared as hydrolysate from the core region of the LPS. Both the hydrolysates were examined by paper chromatography when the same sugars were obtained.

PMR spectrum of F-1 fraction contained three methyl doublets merged into two and accounting three C-5 methyl groups - two from L-rhamnoses (δ 1.27) and one from fucosamine (δ 1.29). The signal at δ 4.97 (2H) indicated two axial anomeric protons corresponding the β-linked L-rhamnose. The peak at δ 5.05 (1H) was probably due to the equatorial anomeric protons corresponding to β-linked D-glucose and the one at 5.20 (1H) was for the equatorial anomeric proton of α-L-fucosamine. In addition, the whole spectrum integrated for 33 protons, in conformity with the tetrasaccharide repeating unit.

Carbon-13 NMR spectrum of F-1 fraction displayed a signal at δ 19.03 corresponding to three CH_3 carbons from two L-rhamnoses and one L-fucsosamine. The signal at δ 104.23 corresponded to three β-glycosides – two β-L-rhamnosides and one β-D-glucoside. The signal at δ 103.11 was probably for the anomeric carbon of α-L-fucosamine moiety. One carbon signal at δ 59.0 (CHNH-) and two carbons each at δ 80.46 and 80.29 (non-anomeric ^{13}C involved in glycosidic linkage) clearly favoured a tetrasaccharide repeating unit. Other signals at δ 74.91 (1C), 74.03 (2C), 73.91 (2C), 72.50 (3C), 71.80 (2C) and 71.62 (2C) were obviously for C-2, C-3, C-5 and C-6 carbons respectively.

The polysaccharide F-1 was methylated, first by the Hakomori procedure[9] and then by the Kuhn method[10]; the absence of hydroxyl absorption in the IR spectrum of the product indicated complete methylation. Analysis of the alditol acetates of the product by GLC showed peaks for 3,4-dimethyl-L-rhamnose, 2,4-dimethyl-L-rhamnose, 2,4-dimethyl-D-glucose and probably 3,4-dimethyl-L-fucosamine. The results of methylation analysis with possible linkages are shown in Table1.

Oxidation of F-1 fraction with sodium metaperiodate[11], showed that half of the L-rhamnoses and D-glucose moieties survived oxidation. All these experiments suggest structure (I) as one of the possibilities for the repeating unit of O-specific F-1 fraction of the polysaccharide of *P. aeruginosa* Habs Type 1. Other sequences of sugars may also be possible.



(I)

Experimental procedure

General method

Paper chromatography was performed on Whatman No. 1 papers using 4:1:5 1-butanol-acetic acid-water (upper layer) as solvent; the spray reagent used was alkaline silver nitrate. All solvents were distilled before use, and all evaporations were conducted at 50° unless statedotherwise. Optical rotations were measured with a Perkin-Elmer Model 241 MC spectropolarimeter. Paper electrophoresis was carried out with a Shandon high voltage electrophoresis model L24 using buffer. Backman ultracentrifuge model L5 65 was used for preparative work.

Gas liquid chromatography (GLC) was performed with a Hewlett-Packard model 5730 A gas chromatograph fitted with a flame ionisation detector and a 3380 A electronic integrator. Columns used were: glass (1.83 m × 6 mm) packed with (A) 3% of ECNSS-M on gas Chrom Q (100-120 mesh), (B) 1% OV-225 on Gas Chrom Q (100-120 mesh) and (C) 3% OV-225 on Gas Chrom Q (100-120 mesh). All GLC analyses were conducted at 185° for unmethylated sugars and at 170° for methylated sugars by converting the sugars into their alditol acetates. Retention times of partially methylated alditol acetates were measured using 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol as reference.

Table 1 — Properties and Sugar Composition of F-1 and F-2 Fractions

	$[\alpha]_D$	Total sugar	Precipitation with homologous Antiserum	Sugar Composition (mol %)		
				Rhamnose	Glucose	Fucosamine
LPS			+ +	41.5	36.6	21.9
F-1	+ 66.6°	90	+ + +	49.4	28.9	21.7
F-2	+ 30.0°	92	+	26.3	51.7	23.0
Periodate oxidised F-1			–	55.2	44.8	–

PMR and ^{13}C NMR spectra were recorded in D_2O at 80°C on a 500 MHz FT-NMR instrument at the Tata Institute of Fundamental Research, Bombay.

Growing bacteria to get dry cells

Pseudomonas aeruginosa Habs Type 1 was grown on a nutrient medium containing 0.5% sodium chloride, 1% Lab lemco powder, 1% peptone and 1.5% agar, at *p*H 7.5 in Roux bottle each containing 150 ml of the medium. The bacteria was incubated at 37° for 48 hr. The growths were harvested with normal saline and the bacteria was killed with 5% phenol (w/v) after removal of the medium particles by filtration through cotton. The cell suspension was centrifuged at 12,000 rpm and washed twice with saline and twice with acetone and then kept overnight *in vacuo* to ger the dry cells.

Isolation of lipopolysaccharide from dry cells of P. aeruginosa

Dry cells (20 g) of bacteria were suspended in water (350 ml) at 65-68° and to this was added 90% phenol (350 ml) preheated at 65-68° dropwise with vigorous stirring. The mixture was kept at 65° for 15 min with stirring and cooled to 10° in an ice-bath. The emulsion was centrifuged at 3000 rpm for 45 min which resulted in the formation of three layers: a water layer, a phenol layer and an insoluble residue at the phenol/water interphase. The water layer was separated. The phenol layer and the insoluble residue were treated at 65-68° with water (350 ml) as described above. The combined water layer was dialyzed for 3 days to remove phenol and low molecular weight bacterial substances. Slightly opalescent dialyzed solution was freeze-dried to give lipopolysaccharide (LPS) as a fluffy powder.

Removal of nucleic acid and purification of LPS

A 3% solution of LPS (1 g) in water was ultracentrifuged at 40,000 rpm for 4 hr. The LPS settled at the bottom of the tube while nucleic acid remained in solution. The crude LPS thus obtained was washed 2 or 3 times with water, dissolved in water, and again centrifuged at 1000 rpm in order to remove extraneous materials. The supernatent LPS solution was lyophilized (yield 430 mg).

The LPS thus obtained was further purified by passing it in 100 mg portions through a column of Sephadex G-100 using pyridine - acetic acid - water (4:10:1000) as eluent, when a broad band was obtained. The solution was lyophilized to give the pure product, yield 77 mg.

Isolation and purification of PS

The LPS was dissolved in 1% acetic acid (2 mg/ml) and heated at 100° for 2 hr. The lipid portion was separated by centrifugation. The supernatant solution was lyophilized to get the crude polysaccharide, which was purified by passing through a column of Sephadex G-100 using pyridine — acetic acid — water (4:10:1000) as eluent and 80 fractions (5 ml each) were collected. The fractions were automatically monitored with a Waters Associates differential refractometer model 403 fitted with an Omni-Scribe recorder.

The pure polysaccharide fractions F-1 (20 mg) and F-2 (30 mg) were collected. F-1 had $[\alpha]_D$ + 66.6° (*c*, 0.7, water) and F-2 had $[\alpha]_D$ + 30° (*c*, 0.8, water).

Acid hydrolysis of polysaccharides

Polysaccharide fractions F-1 and F-2 (2 mg each) were hydrolysed with 3*N* hydrochloric acid (2 ml) at 100° for 6 hr in sealed tubes. The acid was removed by repeated evaporation of the water solution under reduced pressure and the traces of acid were finally removed by drying *in vacuo* over KOH. The product was examined by paper chromatography and by GLC as its alditol acetate[12]. The results are shown in Table 2.

Methylation analysis of polysaccharides

The polysaccharides F-1 and F-2 (10 mg each) were dissolved in dry dimethyl sulfoxide (3 ml) by stirring overnight in a serum vial. To the clear solution, under N_2 atmosphere, was added methylsulfinyl carbanion[9] (3 ml), and the mixture was stirred for 1 hr, and kept overnight. The vial was then cooled in an ice-bath, methyl iodide (3 ml) was added, and the mixture stirred for2 hr. The vial was opened, the excess of methyl iodide removed by passing N_2 through the solution, and

Table 2—GLC Analysis of Fully Methylated F-1 Fraction

Methylated sugars (as alditol acetates)	Retention time (min)		Mol (%)	Linkage
	Column A	Column C		
3,4-Rhamnose	0.92	0.87	23.5	→2)-Rha*p*(1→
2,4-Rhamnose	0.99	0.94	28.7	→3)-Rha*p*(1→
2,4-Glucose	5.10	4.21	29.2	→3)-Glc*p*(1→
3,4-Fucosamine			18.6	$FucNH_2$(1→
2,3,4,6-Glucose	1.00	1.00		

the mixture was dialysed against distilled water for 2 days. The solution from the dialysis bag was lyophilised, to afford methylated F-1 and F-2. The material was remethylated by the Kuhn method[10]. The permethylated F-1 and F-2 fractions of the polysaccharide, were hydrolysed, first with 2*M* trifluoroacetic acid for 2 hr on a boiling water-bath, and then with 3*N* hydrochloric acid for 6 hr at 100°. The alditol acetates were prepared from the hydrolyzate and examined by GLC (columns A and B). The results are summarised in Table 1.

Oxidation of PS F-1 with sodium metaperiodate[11]

To a 0.05% solution of PS F-1 (8 ml) was added 0.2 *M* sodium metaperiodate (2 ml), and the mixture kept in the dark for 48 hr at 5°. The excess periodate was decomposed by adding excess of ethylene glycol (0.5 ml), the mixture was kept for 3 hr and dialysed against distilled water. The solution inside the bag was concentrated to a small volume, $NaBH_4$ (20 mg) was added, and kept for 4 hr at room temperature. Excess borohydride was destroyed by adding acetic acid and the solution dialysed. The dialysed product was freeze-dried and hydrolysed with 3*N* HCl for 6 hr. The alditol acetate prepared from it showed, on GLC, L-rhamnose and D-glucose in the ratio 1:1.

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Structure of the Oligosaccharides Isolated from *Acacia Catechu* (Khair) Gum Polysaccharide

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A water soluble polysaccharide has been isolated from the *Acacia catechu* gum. By exclusion chromatography the material has been fractionated and homogeneity of the product checked by HPLC and ultracentrifugal analysis. The polysaccharide on hydrolysis gives galactose, arabinose, rhamnose and glucuronic acid Partial hydrolysis of the polysaccharide furnishes one di, one tri- and one hexa-saccharides. Hydrolysis of these oligosaccharides furnishes only galactose. Periodate oxidation and methylation analysis of the di- and tri-saccharides indicate the presence of 1 → 3 linked galactose units. However, the hexasaccharide has a branched structure and the side chains of galactose are joined to the 1 → 3 linked galactose main chain by 1 → 2 linkage. Structure of such a hexasaccharide has not been reported earlier.

The gum from *Acacia catechu* (series Vulgares) is of good quality and is regarded as a best substitute for the gum isolated from *A. arabica* (series Gummiferae). It occurs in pale yellow tears and gives thicker and better mucilage than *A. arabica* gum. Analytical studies of *A. hebeclada, A. kirkii, A. newbrownii, A. reficiens* (series Gummiferae) and *A. erubescens, A. fleckii, A. senegal, A. mellifera* spp. *mellifera* and *A. mellifera* spp. *detinens* (series Vulgares) by Anderson and Farquar[1] lend support for the chemotaxonomic differences between the Gummiferae and Vulgares. The gum from *A. arabica* contains a complex and highly branched galactan framework containing 1 → 3 and 1 → 6 linkages. Most of the D-galactose residues are 3,6-di-O-substituted by uronic acid residues and arabinose containing side chains[2].

The present investigations were undertaken to unravel the complete structure of the polysaccharide of *A. catechu* to see its similarity with the gums of Vulgares series and differences with the gums of Gummiferae series. Isolation of the aldobiouronic acid 6—O—β—D—glucopyranosyluronic acid —D—galactose from the gum of *A. catechu* has been reported earlier[3]. The characterization of oligosaccharides isolated from the partial hydrolysis of *A. catechu* gum polysaccharides is described in this paper.

Gum tears, after mechanical separation from adhering pieces of bark, were crushed to powder which was dissolved in water and centrifuged to remove water-insoluble impurities. The centrifugate after acidification with acetic acid (*p*H 3.5-4) was poured into ethanol to precipitate the polysaccharide. The polysaccharide was purified by fractional precipitation method using different concentrations of ethanol and deionised by passing through the columns of Duolite C-25 and Duolite A-7 ion exchange resins. The polysaccharide was further purified by exclusion chromatography by passing it through Sephadex G-50 and G-100 column (Fig. 1). The fractions of the major peak were pooled together, concentrated and polysaccharide was precipitated by the addition of ethanol. Purity of this polysaccharide was checked by HPLC using μ-Bondagel E-300 and E-1000 columns (Fig. 2). Purity of the polysaccharide was further checked by ultracentrifugal analysis (Fig. 3). Sedimentation constant of the polysaccharide was found to be 6.86 which corresponded to a molecular weight of 40,010.

The pure polysaccharide had ash content 0.28%, nitrogen 0.0% methoxyl, 1.7%, anhydrouronic acid, 3.8%, and optical rotation $[\alpha]_D^8 - 30°$ (*C*, 0.16% in acetate buffer *p*H 4.8). Complete hydrolysis of the gum followed by GLC of its sugar alditol acetates prepared by the method of Kakuta and Misaki[4], showed the presence of galactose (64.57%), arabinose (24.21%), rhamnose (6.72%) and glucuronic acid (4.03%). The presence of 4—O—Me-glucuronic acid

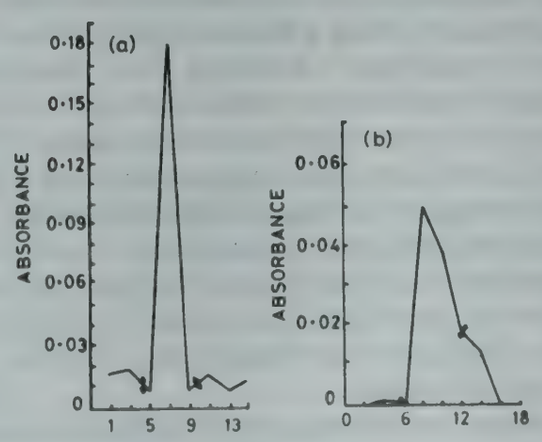


Fig. 1—Parent polysaccharide run on sephadex: (a) G-50); (b) G-100

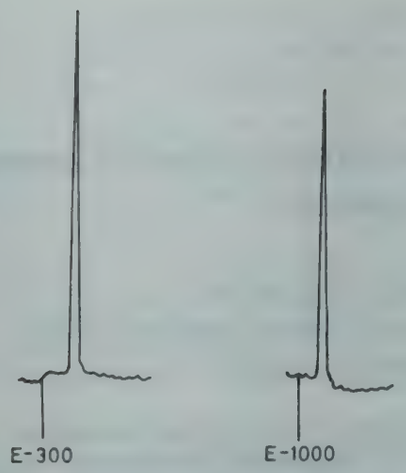


Fig. 2—HPLC of parent polysaccharide

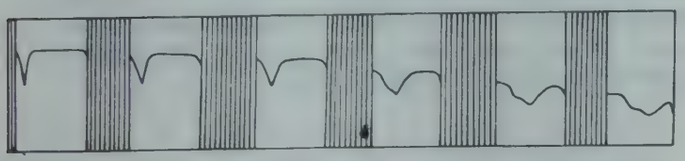


Fig. 3—Ultracentrifuge run of parent polysaccharide

Table 1—PMR and ^{13}C NMR Spectral Data of Gum Polysaccharide

PMR		^{13}C NMR	
Signal (δ, ppm)	Configuration assignment	Signal (δ, ppm)	Configuration assignment
4.1	β-Gal_p5	97.93	β-Gal_p6
5.7	α-Ara_f7	100.18	α-Ara_f8
5.3	α-Rha_p9	100.95	α-Rha_p9
4.5	β-Glu_pA^5	175.68	β-Glu_pA^{10}

(~1.7% of total glucuronic acid) was indicated by 4—O—Me-glucose peak. Anomeric configuration of the above constituent sugars was determined by PMR and ^{13}C NMR spectra of the polysaccharide in D_2O (Table 1).

Partial hydrolysis

A. catechu gum polysaccharide on hydrolysis with dil. sulphuric acid (0.1 *N*, 4 hr) furnished a mixture of oligosaccharides along with monosaccharides consisting of D-galactose, L-arabinose and L-rhamnose. The oligosaccharides were isolated from the mixture by preparative paper chromatography using Whatman No. 3 mm sheets. Four oligosaccharides were isolated in pure state. The degree of polymerisation[11] of three oligosaccharides corresponded to di-, tri-, and hexa-saccharides. D.P. of the fourthe oligosaccharide could not be determined due to paucity of the compound. All the oligosaccharides on hydrolysis afforded galactose only.

The nature and sequence of glycosidic linkages between galactose residues were determined both by periodate oxidation and methylation analysis.

Structure of Oligosaccharide-I

It was a disaccharide (32 mg), $[\alpha]_D^{26}$ + 30° to 59.6° (43 hr, equil), m.p. 142-46°(d), R_{gal} 0.54 (solvent S_1), and found to be chromatographically pure. During oxidation with sodium metaperiodate[12], it consumed 3.1 moles of periodate. After complete methylation[13,14], hydrolysis, reduction, acetylation and GLC (condition C_1) it furnished peaks corresponding to one mole each of 2, 4, 6-tri-O-methyl-D-galactose and 2, 3, 4, 6-tetra-O-methyl-D-galactose. Methylation analysis showed the presence of 1 → 3 linkage between the two galactose moieties. The consumption of 3.1 moles of periodate (theoretically required 3.0 moles) by the disaccharide also lent support to the presence of 1 → 3 linkage between the two galactose units. On the basis of above studies, the dissaccharide was designated as 3—O—β—D—galactopyranosyl-D-galactose.

Structure of oligosaccharide-II

It was a trisaccharide (65 mg), $[\alpha]_D^{26}$ + 39.0°, m.p. 98-102°(d), R_{gal} 0.30 (solvent S_1), and was found homogeneous by paper chromatography. On oxidation with sodium metaperiodate[12], it consumed 2.90 moles of the reagent. It was methylated[13,14], hydrolysed, reduced and acetylated. The GLC analysis of its alditol acetates (condition C_2) gave peaks corresponding to 1 mole of 2, 3, 4, 6-tetra-O-methyl-D-galactose from non-reducing end and two units of 2, 4, 6-tri-O-methyl-D-galactose from the 1 → 3 linked units of galactose moiety. Consumption of 2.90 moles of periodate (theoretically required 3.0 moles) corroborate the methylation results. Thus, the trisaccharide was designated as O—β—D—galactopyranosyl-(1 → 3)—O—β—D—galactopyranosyl-(1 → 3)—D—galactopyranose.

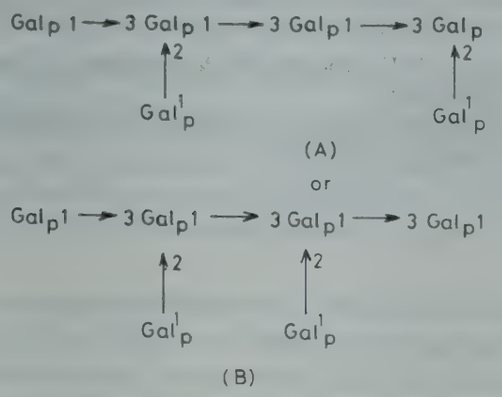
Structure of oligosaccharide-III

The oligosaccharide-III (17 mg), $[\alpha]_D^{26}$ + 45°, m.p. 146-150°(d), had R_{gal} 0.18 (solvent S_1). Further work could not be carried out due to paucity of the material.

Structure of oligosaccharide-IV

It was a hexasaccharide (150 mg), $[\alpha]_D^{26}$ −11.5°, m.p. 138-140°(d), R_{gal} 0.05 (solvent S_1), and was found homogeneous by paper chromatography. During oxidation with sodium metaperiodate[12] it consumed 6.93 moles of oxidant (theoretically required 7.0 moles). It was completely methylated[15,14], hydrolysed, reduced and acetylated. The GLC analysis of its alditol acetates (condition C_2) gave peaks corresponding to 2, 3, 4, 6-tetra-O-methyl-D-galactose (3 moles), 2, 4, 6-tri-O-methy-D-galactose (1 mole) and 4, 6-di-O-methyl-D-galactose (2 moles). Methylation analysis indicated the hexasaccharide to have a

branched chain structure in which tetra-O-methylgalactose forms the non-reducing end (—C_1 linkage), tri-O-methylgalactose shows the presence of 1→3 linkages and di-O-substituted galactose demonstrates that the main chain of (1→3) linked galactose units is joined by 1→2 linked side chains of galactose moiety. Accordingly, one of the tentative structures **A** and **B** may be assigned for the hexasaccharide which is also in agreement with periodate oxidation results. This structure of hexasaccharide is being reported for the first time.



Experimental Procedure

Gum tears were obtained from the forests of Mohand, Dehra Dun (U P India).

General methods of analysis

Solutions were concentrated at or below 40° in rotary evaporators under reduced pressure. All melting points are uncorrected. Paper chromatography was carried out on Whatman No. 1 and 3 mm paper sheets using ethyl acetate-acetic acid-butanol-water (4:3:2:2 v/v) as solvent system (S_1). Detection was effected with acetonic silver nitrate-alcoholic sodium hydroxide[16] (designated as R).

Exclusion chromatography was performed on Sephadex G-50 and G-100 columns (54 × 0.5 cm) using 0.02% aq. solution of sodium azide as eluant. Fractions (2 ml each) were collected and monitored with anthrone-sulphuric acid reagent at 660 mμ (ref. 17). High performance liquid chromatography (Water Associate Inc) was done on μ-Bondagel E-300 and E-1000 columns using water as the mobile phase. GLC analysis of the partially methylated alditol acetates was carried out using CIC gas chromatograph (FID) on ECNSS-M (3%) on Gas chrom Q (100-120 mesh) packed into a 5′ × 1/8″ stainless steel column under the following conditions: (C_1) column temperature 170-200°, programmed (2°/min), nitrogen flow rate 60 ml/min, and (C_2) column temperature 190°, nitrogen flow rate 35 ml/min. PMR and ^{13}C NMR spectra of the polysaccharide were recorded in D_2O on Perkin-Elmer R-32 and Bruker W.M.-400 spectrospin NMR spectrophotometers, respectively. Ultracentrifugal analysis of the polysaccharide (in 0.1 *M* acetate buffer) was performed on a Beckman Spinco Model E ultracentrifuge at 68000 r.p.m. at 25°.

Partial hydrolysis

Gum polysaccharide (7 gm) was heated on a steam-bath with sulphuric acid (0.1 *N*, 500 ml) for 4 hr. The hydrolysate was cooled, neutralised ($BaCO_3$) and filtered. The residue was repeatedly washed with water. The combined filtrate was passed through a column of Duolite C-25 and concentrated to a syrup (6.640 g). Oligosaccharides were isolated by preparative paper chromatography on Whatman No. 3 mm sheets using S_1 as irrigant and R as a spray reagent.

Complete hydrolysis

Oligosaccharides (4-10 mg) were treated with sulphuric acid (2*N*) on a steam-bath for 20-22 hr to achieve complete hydrolysis.

Periodate oxidation

Samples (5-10 mg) of the various oligosaccharides were subjected to periodate oxidation with sodium metaperiodate solution (10^{-1} *M*, 5 ml) and the consumption of periodate was determined by the spectrophotometric method using phenolphthalein as indicator at 555 nm[12].

Methylation analysis of oligosaccharides

The disaccharide (19 mg) was methylated by the procedure of Jansson *et al.*[15] followed by Purdies[14] method using methyl sulphinyl carbanion and silver oxide-methyl iodide. The trisaccharide (43 mg) and hexasaccharide (110 mg) were methylated by the methods of Hakomori[12] and Purdies[14] using sodium hydride-dimethyl sulphoxide and silver oxide-methyl iodide. Each methylated oligosaccharide was recovered by chloroform extraction. After evaporation of the chloroform extracts to dryness, the residues were treated with formic acid (90%, 10 ml) for 1 hr on a steam-bath, the solutions evaporated and treated with aq. sulphuric acid (0.13 *M*, 15 ml) for 16 hr on a steam-bath.

Alditol acetates[15]

The hydrolysed sugars were neutralised with barium carbonate and reduced with sodium borohydride. After 4 hr the solution was acidified with acetic acid (*p*H 3.5) and co-distilled with methanol. The resulting alditols were acetylated by refluxing with acetic anhydride and pyridine (1:1, v/v) for 6 hr. The resulting alditol acetates were partitioned between water and methylene chloride and the combined methylene chloride phases concentrated for GLC analysis.

Acknowledgement

The authors are thankful to Mr G P Maithani, Director, Silviculture Research and to Dr K S Ayyar, Officer-in-Charge for their keen interest in this work. One of the authors (A A) is specially thankful to the Ministry of Agriculture for the award of a research fellowship.

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Notes

New Steroidal Heterocycles: Part XX—Total Synthesis of B-Homo-6-oxaestra-1,3,5(10),8,14-pentaen-17-one

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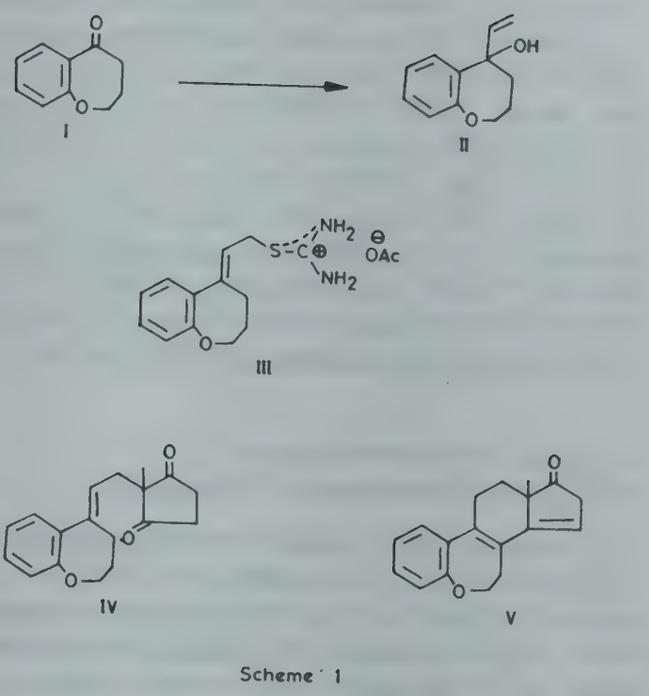
2,3,4,5-Tetrahydro-1-benzoxepin-5-one (I) on treatment with vinylmagnesium bromide affords the vinyl carbinol (II) which on treatment with thiourea and glacial acetic acid yields the isothiuronium acetate (III). Condensation of III with 2-methylcyclopentane-1,3-dione leads to the 8,14-C-secosteroid (IV), which on cyclodehydration with PTS furnishes the title compound (V).

Though considerable literature is available on oxasteroids[1,2], no work seems to have been reported on B-homo-6-oxaestron derivatives. Herein we report a total synthesis of B-homo-6-oxaestra-1,3,5(10),8,14-pentaen-17-one (V) starting from 2,3,4,5-tetrahydro-1-benzoxepin-5-done[3] (I).

The starting material I, prepared as per literature procedure[3], on treatment with vinylmagnesium bromide under Normant reaction conditions[4] in dry tetrahydrofuran (THF) gave 5-hydroxy-5-vinyl-2,3,4,5-tetrahydro-1-benzoxepin (II) as a thick yellow liquid in 85% yield. It was rapidly purified by chromatography over neutral alumina. The carbinol (II) reacted smoothly with thiourea and glacial acetic acid[5] affording the expected 2,3,4,5-tetrahydrobenzooxepinylidene ethylisothiuronium acetate (III) in 65% yield (Scheme 1).

Condensation of III with 2-methylcyclopentane-1,3-dione[6] in 50% (v/v) aq. ethanol under reflux furnished after chromatographic purification of the brownish gum, 8,14-seco-B-homo-6-oxaestra-1,3,5(10),9(11)-tetraene-14,17-dione (IV) as a yellow gummy material in 78% yield. Cyclodehydration of IV with *p*-toluenesulphonic acid in refluxing dry benzene led to the anticipated B-homo-6-oxaestra-1,3,5(10),8,14-pentaen-17-one (V) as a greenish yellow gum in 72% yield. Catalytic or ionic hydrogenation[7] of V, was unsuccessful.

The recorded temperatures are uncorrected.



Scheme 1

5-Hydroxy-5-vinyl-2,3,4,5-tetrahydro-1-benzoxepine (II)

To a stirred suspension of vinylmagnesium bromide (from 20 ml of vinyl bromide and 3.5 g magnesium turnings) in dry THF, cooled to −20° was added dropwise a solution of I[3] (3.2 g) in dry THF, precooled to −20°C under N_2 atmosphere. The reaction mixture was slowly brought to room temperature and then refluxed for 4 hr. The Grignard complex was decomposed with ice cold saturated aq ammonium chloride and the yellowish brown product extracted with ether (3 × 100 ml). The ether extract was dried and evaporated under reduced pressure to furnish the crude II (3.5 g), which was immediately purified by chromatography over neutral alumina (100 g), because of its tendency to decompose during distillation under reduced pressure (10^{-4} torr). Elution with methylene chloride afforded a fairly pure sample of II (3.1 g, 85%). All attempts to induce solidification failed; IR (smear): 3500-3200 (br, OH), 1640 (C=C), 1600-1560, 1500 cm^{-1} (aromatic C=C); PMR ($CDCl_3$): δ 1.4-2.0 (*m*, 2H, O-CH_2−CH_2−CH_2), , 2.6 (*t*, 2H, O−CH_2−CH_2−CH_2), 3.4 (*br*, 1H, OH, exchangeable with D_2O), 4.0 (*t*, 2H, O−CH_2−CH_2−CH_2). The complex multiplet of 3H intensity observed at δ 4.7-6.3 (C*H*=CH_2) was comparable with the expected *ABC* pattern for the vinylic protons. The aromatic protons appeared at δ 6.6-7.4 as a multiplet; MS: *m/z* (%) 190 (12), 172 (10), 163 (10), 160 (15), 148 (100), 147 (80), 132 (42) and 104 (18).

2,3,4,5-Tetrahydrobenzoxepinylidene ethylisothiuronium acetate (III)

To a stirred mixture of II (2.8 g) and thiourea (1.1 g) cooled to 0-5°C was added gl acetic acid (12 ml). The resulting mixture was slowly brought to room

temperature and stirred for 12 hr. Dry ether (80 ml) was slowly added to the reaction mixture and the precipitated solid filtered off and washed several times with dry acetone (5 × 20 ml) to furnish pure III (3 g, yield 65%) as a white amorphous solid, m.p. 138-40°; IR (KBr): 3300 (br, NH), 1560 cm^{-1} (acetate anion); PMR (DMSO-d_6): δ 1.7 (*s*, 3H, $OCOCH_3$), 1.8-2.5 (*m*, 4H, $O-CH_2-CH_2-CH_2$), 3.7 (*d*, 2H, $=CH-CH_2-S$), 4.0 (*t*, 2H, $O-CH_2-CH_2-CH_2$), 5.6 (*t*, 1H, *J* = 7 Hz, olefinic proton), 6.6-7.1 (*m*, 8H, ArH and amino protons); MS: *m/z* (%) 249 (25), 206 (100), 173 (98), 172 (75), 158 (70), 145 (73) and 131 (65) (Found: C, 58.6; H, 6.52. $C_{15}H_{20}N_2O_3S$ requires C, 58.4; H, 6.5%).

8,14-*Seco*-*B*-*homo*-6-*oxaestra*-1,3,5(10),9(11)-*tetraene*-14,17-*dione* (*IV*)

Compound (III, 1 g) and 2-methylcyclopentane-1,3-dione (0.34 g) were refluxed in 50% (v/v) aq ethanol (100 ml) for 5 hr. The solvent was removed under reduced pressure, the residue extracted with ether (4 × 25 ml) and the ether extract washed successively with saturated solutions of sodium bicarbonate (2 × 25 ml) and sodium chloride (2 × 25 ml). Evaporation of the dried ether layer afforded a pale yellow thick gum (0.9 g) which on chromatography over neutral alumina (30 g) gave from benzene-hexane (1:1) eluates (600 ml), pure IV (0.7 g, 78%) as a yellow gum. All attempts to solidify the gum failed. Attempted distillation *in vacuo* was not successful; IR ($CHCl_3$): 1760, 1730 (2,2-disubstituted cyclopentane-1,3-dione moiety), 1600-1450 cm^{-1} (aromatic C = C); PMR ($CDCl_3$): δ 1.1 (*s*, 3H, C_{18}-methyl), 1.8 (*m*, 2H, $O-CH_2-CH_2-CH_2$), 2.5 (*t*, 2H, $O-CH_2-CH_2-CH_2$), 2.7 (*s*, 4H, $CO-CH_2-CH_2-CO$), 3.5 (*d*, 2H, *J* = 10 Hz, C_{12}-H_2), 4.0 (*t*, 2H, $O-CH_2-CH_2$), 5.2 (*t*, 1H, olefinic proton) and 6.6-7.1 (*m*, 4H, Ar − H); MS: *m/z* (%) 284 (13), 173 (100), 172 (60), 158 (40), 157 (38), 145 (80), 144 (50) and 131 (75).

B-*Homo*-6-*oxaestra*-1,3,5(10),8,14-*pentaen*-17-*one* (*V*)

A solution of IV (0.57 g) in dry thiophene-free benzene (70 ml) containing *p*-toluenesulphonic acid (PTS) (0.04 g) was refluxed for 15 min employing Dean-Stark water separator. The reaction mixture was cooled and poured onto crushed ice (50 g). The organic layer was separated. The aqueous layer was extracted with benzene (2 × 30 ml) and the combined benzene extract successively washed with saturated solutions of sodium bicarbonate (2 × 15 ml) and sodium chloride (2 × 20 ml). Evaporation of the solvent gave a yellowish brown gum (0.4 g) which on chromatographic purification over silica gel (50 g) furnished from benzene-hexane (3:2) (100 ml) eluates pure V (0.38 g, 72%) as a greenish yellow gum. All attempts to solidify the gum failed; IR ($CHCl_3$): 1725 (C = O), 1660-2440 cm^{-1} (aromatic C = C); PMR ($CDCl_3$): δ 1.1 (*s*, 3H, C_{18}-methyl), 1.2-2.8 (*m*, 6H, methylene protons at C-11, C-12 and C-16), 3.2 (*t*, 2H, $O-CH_2-CH_2$), 4.0 (*t*, 2H, $O-CH_2-CH_2$), 6.3 (*t*, 1H, olefinic proton) and 6.7-7.2 (*m*, 4H, aromatic protons); MS: *m/z* (%) 266 (100), 265 (32), 238 (55) and 237 (10) (Found: C, 81.5; H, 6.8. $C_{18}H_{18}O_2$ requires C, 81.2; H, 6.8%).

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A Simple One-Step Synthesis of Steroidal Lactams from Ketones of Spirostane & Cholestan Series

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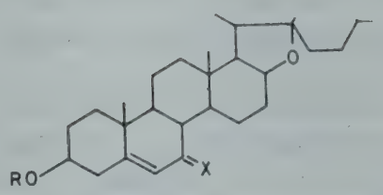
The steroidal ketones (25*R*)-7-oxo-5-spirosten-3β-yl methyl ether (**1a**), (25 *R*)-7-oxo-5-spirosten-3β-yl ethyl ether (**1b**), 7-oxo-5-cholestene (**2a**) and 7-oxo-3,5-cholestadiene (**3a**) afford the respective lactams (**4, 5 6** and **7**) on treatment with hydroxyl amine-O-sulphonic acid (HOSA) in hot formic acid. The identity of the lactams **4, 5, 6** and **7** have been confirmed by comparison with the lactams obtained by Beckmann rearrangement of the ketoximes (**1e, 1f, 2c, 3c**) and spectral analysis.

Beckmann rearrangement of steroidal ketoximes and Schmidt reaction of steroidal ketones are the usual methods to obtain steroidal lactams[1]. Synthesis of lactams from alicyclic ketones has been reported earlier by Colan and Fung[2] by the action of hydroxylamine-O-sulphonic acid (HOSA) is hot formic acid. We now report the preparation of steroidal lactams by the above method.

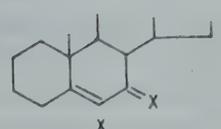
The starting compounds, methyl and ethyl ethers of (25 *R*)-5-spirosten-3β-ol were prepared following Narayan and Iyer's method[3]. Chromic acid oxidation of these ethers in gl. acetic acid afforded the corresponding 7-oxo derivatives (**1a** and **1b**).

The steroidal ketones (**1a, 1b, 2a** and **3a**) were treated with HOSA in hot formic acid (60-70°) under nitrogen atmosphere for 2 hr when the corresponding lactams (**4, 5, 6** and **7**; Table 1) were obtained in good yields (60%). The action of HOSA in formic acid at room temperature (30°) for 30 min under nitrogen atmosphere on **1a, 1b, 2a** and **3a** gave the respective oxime-O-sulphonic acids (**1c, 1d, 2b** and **3b**) [IR: 3400 (OH) and 1630 cm^{-1} (C=N)] which were converted into lactams (**4, 5, 6** and **7**) during chromatography over neutral alumina.

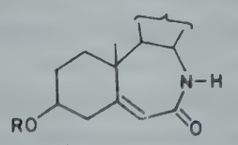
The IR spectra of the lactams (**4, 5, 6** and **7**) exhibited absorption bands around 3350 (OH) and 1500 cm^{-1} (−C=CO−C−NH−). The UV spectra of **4, 5** and **6** showed absorption maxima around 220 (log ε 4.1) characteristic of α,β-unsaturated lactams[4] and of **7** at 270 nm (log ε 4.2) for dienone lactams[5]. The PMR spectra of the lactams displayed signals around δ 6.95 (*m*, 1H for NH, exchangeable with D_2O), 4.45 (*m*, 1H, $W_{1/2}$ = 12 Hz, 3α−H)[6], 5.55 (*s*, 1H, vinylic proton) and 3.3 (*m*, 1H, 8β−H).



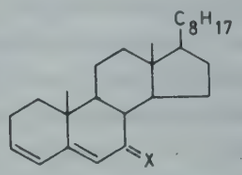
	R	X
1a	CH_3	O
b	C_2H_5	O
c	CH_3	$NOSO_3H$
d	C_2H_5	$NOSO_3H$
e	CH_3	NOH
f	C_2H_5	NOH



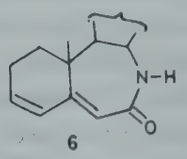
	X
2a	O
b	$NOSO_3H$
c	NOH



4 R=CH_3 5 R=C_2H_5



	X
3 a	O
b	$NOSO_3H$
c	NOH



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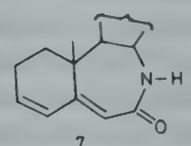

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Table 1—Characterization of the Various Compounds Prepared

Compd	m.p. °C	Mol. formula	Found (%) (Calc.)		
			C	H	N
1a	185-87	$C_{28}H_{42}O_4$	75.2	9.4	—
			(76.0	9.5	—)
1b	177-79	$C_{29}H_{44}O_4$	75.3	9.5	—
			(76.3	9.6	—)
1c	195-97	$C_{28}H_{43}NO_7S$	61.5	7.8	2.4
			(62.5	8.0	2.6)
1d	160-62	$C_{29}H_{45}NO_7S$	62.2	8.1	2.1
			(63.2	8.2	2.2)
2b	109-10	$C_{27}H_{45}NO_4S$	66.9	9.4	2.9
			(67.6	9.4	2.9)
3b	150-52	$C_{27}H_{43}NO_4S$	67.0	9.0	2.9
			(67.9	9.0	2.9)
4	200-202	$C_{28}H_{43}NO_4$	72.4	9.2	3.0
			(73.5	9.4	3.1)
5	175-77	$C_{29}H_{45}NO_4$	72.9	9.5	3.0
			(73.9	9.6	3.0)
6	209-10[8]	$C_{27}H_{45}NO$	80.1	11.2	3.3
			(81.2	11.3	3.5)
7	170-71[5]	$C_{27}H_{43}NO$	80.5	10.8	3.5
			(81.6	10.8	3.5)

The above lactams (**4, 5 6** and **7** were also prepared by the Beckmann rearrangement of the ketoximes (**1e, 1f, 2c** and **3c** using PTS in pyridine followed by column chromatography over neutral alumina, during which the oxime tosylates underwent

rearrangement to lactams (**4, 5, 6** and **7**). The lactams obtained by the above two methods were identical in all respects (IR, m.m.p. and co-TLC). Their spectral data were comparable with those of (25*R*)-7-oxo-B-homo-5-spirosten-3β-yl acetate prepared by Singh *et al.*[7]

Melting points are uncorrected. IR spectra were recorded in KBr on a Perkin-Elmer 137 spectrophotometer, UV spectra in 95% methanol on a Beckmann Du_z spectrophotometer, and PMR spectra in $CDCl_3$ on a Varian A-60 instrument using TMS as internal standard.

Preparation of 3β-*alkoxy*-5-*spirosten*-7-*ones* (**1a, 1b**): (*A typical experiment*)

To a stirred solution of 3β-alkoxy-5-spirostene (5.4 g) in gl. acetic acid (60 ml) a solution of chromium trioxide (4 g) in 50% AcOH (10 ml) was added during 2 hr maintaining the temperature at 75-80°, and the mixture stirred further for 2 hr at the same temperature. The excess chromium trioxide was destroyed by adding methanol (10 ml) and the acetic acid removed under reduced pressure when a dark semi-solid was obtained. It was extracted with benzene and the benzene layer washed successively with aq. sodium hydroxide solution, dil. HCl and water, dried and solvent removed to give the 7-oxo derivative (**1a** or **1b**). It was recrystallized from ethanol as colourless needles.

Action of HOSA on 7-*oxo derivatives* (**1a, 1b, 2a** *and* **3a**) *in formic acid*

(i) *At* 60-70°: *Formation of the lactams* (**4, 5, 6** *and* **7**)

To a solution of the 7-oxo derivative (1 g) in formic acid (98%, 20 ml) a solution of HOSA in formic acid (0.4 g in 10 ml) was added dropwise and the mixture heated on a water-bath at 60-70° for 2 hr, poured into ice-cold water, extracted with benzene, and the extract worked-up in the usual way to give the respective lactam (0.6 g).

(ii) *At room temperature* (30°): *Formation of oxime* -*O*-*sulphonic acids* (**1c, 1d, 2b** *and* **3b**)

To a solution of the 7-oxo derivative (1 g) in formic acid (98%, 20 ml) HOSA (0.5 g) was added at room temperature during 10 min. After keeping the mixture for 30 min at room temperature (30°), it was poured into ice-cold water, extracted with benzene, and the extract worked up in the usual way to give the respective oxime-O-sulphonic acid (0.8 g).

Preparation of the ketoximes (**1e, 1f**)

To a solution of the ketone **1a** or **1b** (1 g) in ethanol (20 ml) hydroxylamine hydrochloride (2.5 g) and sodium acetate (4.5 g) were added and the mixture was refluxed for 4 hr. The excess of solvent was distilled off and the residue poured into ice-cold water to obtain the oxime **1e** or **1f** (0.8 g) which was recrystallized from ethanol.

Beckmann rearrangement of steroidal ketoximes (**1e, 1f**): *Formation of the lactams* (**4, 5**)

To a solution of the ketoxime **1e** or **1f** (0.8 g) in dry pyridine (10 ml) *p*-toluenesulphonyl chloride (1 g) was added and the mixture kept at room-temperature (30°) for 12 hr and diluted with ice-cold water when the corresponding ketoxime-O-tosylate separated out. It was extracted with ether-$CHCl_3$ (1:1) and the extract washed with dil. HCl and water. Removal of the solvent gave the oxime tosylate as a light brown coloured semi-solid which was absorbed over a column of neutral alumina (30 g, Brockmann grade 1) and eluted with pet. ether (1 litre) and pet. ether-benzene (1:1, 1 litre). While pet. ether eluate gave the unreacted oxime tosylate, pet. ether-benzene (1:1) eluate afforded the lactam **4** and **5**.

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Intermediates for Steroid Synthesis: Alkylation of Cyclic 1,3-Diketones with a Furan Derivative

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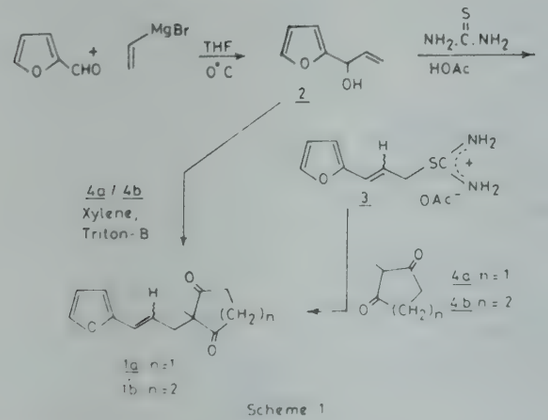
The potential intermediates for steroids **1a** and **1b** have been prepared starting from furfural via the Torgov reaction.

Torgov reaction has been extensively used in the synthesis of aromatic and heteroaromatic steroids[1]. As a part of our studies in the synthesis of steroidal derivatives[2], we report herein the synthesis of 2-(3-α-furylprop-2-enyl)-2-methylcyclopentane/cyclohexane-1,3-diones (**1a, b**)—potential synthons for our target molecules.

Reaction of vinylmagnesium bromide with furfural at 0° resulted in the formation of α-furylvinylcarbinol (**2**) as an oil in 64% yield. It was subjected to Torgov reaction by refluxing with 2-methylcyclopentane-1,3-dione (**4a**) in xylene using Triton-B as the catalyst to afford **1a** as an oil in 55% yield. Extension of this reaction to the diketone **4b** afforded **1b** as a solid in 25% yield.

Compound (**2**) being unstable was converted into the stable isothiouronium salt (**3**)[3]. Condensation of **3** with **4a** proceeded smoothly in aq. ethanol (24 hr stirring) to afford **1a** in 46% yield. Similar condensation of **3** with **4b** in water at 60° (7 hr) afforded **1b** in 20% yield (Scheme 1).

The stereochemistry about the double bond in compounds **3, 1a** and **1b** could not be ascertained because of the complexity in the olefinic region of their PMR spectra. Reaction of **1a** and **1b** with benzyne is under investigation.



Scheme 1

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1-(α-*Furyl*)*prop*-2-*en*-1-*ol* (**2**)

To a stirred suspension of magnesium (24.3 g, 1 g atom) in dry tetrahydrofuran (200 ml) was added a solution of vinyl bromide (107 g, 1 mol) in THF (200 ml) during 1 hr, gently warmed on a steam-bath for 30 min, cooled in an ice-bath and freshly distilled furfural (35 g, 0.37 mol) in THF (100 ml) added. Stirring was continued for 3 hr and the Grignard complex decomposed with saturated aq ammonium chloride. Extraction with ether, drying and solvent removal gave **2** as a yellow oil (90°/2 mm); yield 29.3 g (64%); IR($CHCl_3$): 3600, 3440, 1620, 890 cm^{-1}; PMR(CCl_4/TMS): δ 3.3 (*bs*, 1H, OH, exchangeable with D_2O) 5.00-5.46 (*m*, 3H, $-CH-OH$ and $=CH_2$) 6.00-6.26 (*m*, 3H, olefinic and furan ring protons), 7.26 (*s*, 1H, α−H of furan ring) (Found: C, 67.5; H, 6.7. $C_7H_8O_2$ requires C, 67.7; H, 6.5%).

2-(3-α-*Furylprop*-2-*enyl*)-2-*methylcyclopentane*-1,3-*dione* (**1a**)

A mixture of vinylcarbinol (**2**, 1.24 g, 0.01 mol), diketone (**4a**, 1.12 g, 0.01 mol), xylene (20 ml) and Triton-B (1 ml) was refluxed under nitrogen for 7 hr. Ether was added and the precipitated diketone **4a** filtered. The filtrate was washed with aq. sodium bicarbonate, water and dried. Removal of solvent and chromatography over silica gel (benzene) gave **1a** as a viscous yellow liquid; yield 1.2 g (55%); UV(ethanol): 270 nm; IR($CHCl_3$): 1760, 1730, 1630, 885 cm^{-1}; PMR($CDCl_3$/TMS): δ 1.12 (*s*, 3H, CH_3), 2.45 (*d*, 2H, CH_2), 2.66 (*s*, 4H, $2\times CH_2$), 5.92-6.37 (*m*, 4H, olefinic and β,γ protons of furan ring), 7.35 (*s*, 1H, α−H of furan ring); MS: *m/z* 218 (M^+) (Found: C, 71.4; H, 6.2. $C_{13}H_{14}O_3$ requires C, 71.5; H, 6.5%).

2-(3-α-*Furylprop*-2-*enyl*)-2-*methylcyclohexane*-1,3-*dione* (**1b**)

It was prepared as **1a** by refluxing carbinol (**2**, 4.96 g, 0.04 mol), diketone (**4b**, 5.04 g, 0.04 mol), xylene (30 ml) and Triton-B (1 ml) for 10 hr. Extraction with ethyl acetate followed by chromatography furnished **1b** as colourless solid; yield 2.3 g (25%); m.p. 85-86°; UV(ethanol): 270 nm;

IR(KBr): 1730, 1700, 890 cm^{-1}; PMR($CDCl_3$/TMS): δ 1.3 (*s*, 3H, CH_3), 1.8-2.15 (*m*, 2H, CH_2), 2.55-2.72 (*m*, 6H, $3 \times CH_2$), 5.7-6.35 (*m*, 4H, olefinic and β,γ protons of furan ring), 7.3 (*s*, 1H, α−H of furan ring); MS: *m/z* 232 (M^+) (Found C: 72.3; H, 6.6. $C_{14}H_{16}O_3$ requires C, 72.4; H, 6.9%).

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Synthesis of Phytol Isomers: 7,11,15-Trimethyl-3-methylenehexadecanol & 3,7,11,15-Tetramethylhexadec-3-en-1-ol & Their Conversion into Vitamin E †

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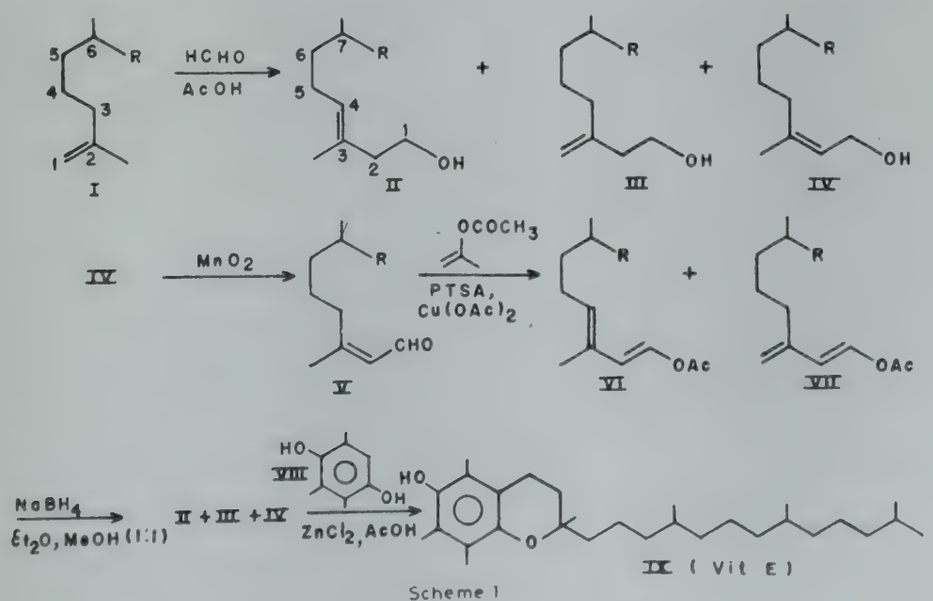
Norphytene on Prins reaction with paraformaldehyde gives phytol isomers from which the title compounds have been isolated by $AgNO_3/SiO_2$ chromatography, and converted into vitamin E.

Phytol and isophytol are the most widely used diterpene alcochols for the synthesis of vitamin E, although other derivatives like phytyl bromide, 1,3-phytadiene, 1,3-dibromophytane and 1,3-phytanediol have also been used. However, there is no mention in literature about the utilisation of phytol isomers (II and III) such as the title compounds. In fact one of the phytol isomers (III), 7,11,15-trimethyl-3-methylenehexadecanol is a naturally occurring diterpene alcohol isolated from tobacco[1], while the other isomer (II), 3,7,11,15-tetramethylhexadec-3-en-1-ol, is unknown. Recently we have described various approaches for the synthesis of norphytene[2] and its isomer for possible use as intermediates in vitamin E synthesis. Our interest in norphytene and its Prins reaction[3] product with formaldehyde as an intermediate for vitamin E synthesis prompted us to isolate the other isomers of phytol and elucidate their structure. The present note describes our results.

Prins reaction on norphytene (I) did not give the phytol IV in 28% yield as reported earlier[3]. Instead the titled diterpene alcohols (II and III) along with a small amount of the phytol IV (Scheme I) was obtained in 55% yield. The homoallylic alcohols II and III were separated on $AgNO_3/SiO_2$ (15%, 1:30 ratio) chromatography and eluted exhaustively with benzene. Thirty fractions of 15 ml each were collected and checked by TLC (solvent:benzene, TLC plate $AgNO_3/SiO_2$, 15%). Fractions 8-20 contained pure II [b.p. 180-82° (bath)/mm] [Found: C, 83.0; H, 13.3. $C_{20}H_{40}O$ requires C, 83.1; H, 13.5%)] and fractions 22-26 contained pure III[1]. Earlier fractions showed the presence of phytol IV. Compounds II and III were characterised by their spectral data [II – IR(neat): 3320, 1040 cm^{-1} (–OH); M^+ 296; PMR(CCl_4): δ 0.85 (*d*, 12H, $4CH_3-CH-$, $J=6$ Hz), 1.6 (*s*, 3H, $\geqslant C-CH_3$), 3.6 (*t*, 2H, $-CH_2-OH$, $J=7$ Hz), 5.2 (*bt*, 1H. olefinic proton); III – IR(neat): 3320 (–OH), 1635, 883 ($\geqslant C=CH_2$) cm^{-1}; M^+ 296; PMR(CCl_4): δ 0.85 (*d*, 12H, 4-CH_3-CH-, $J=6$ Hz), 3.63 (*t*, 2H, $-CH_2-OH$, $J=7$ Hz), 4.78 (*bs*, 2H, $>C=CH_2$)]. Both II and III showed identical mass spectral pattern having major fragments at *m/z* 278 (M – 18), 196 and 123 (base peak) (Chart 2). It therefore seems that compound III isomerises to II during electron impact and thus gives the same fragmentation pattern.



Scheme 1

†NCL Communication No. 4145

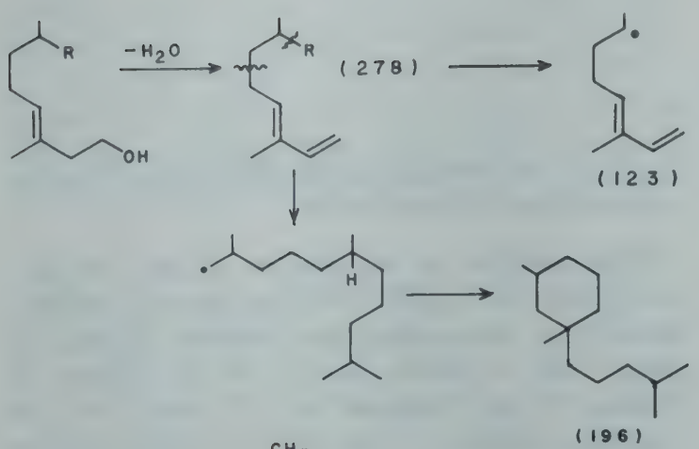


Chart 1

The structures of the alcohols II and III were proved unambiguously by synthesis starting from the authentic phytol IV. The phytol (IV) was oxidized to phytenal (V) using active manganese dioxide[5] in pet. ether which gave *E*- and *Z*-isomers of V in almost quantitative yields; IR(neat): 1670 cm^{-1} (conjugated CHO); PMR(CCl_4): δ 0.85 (*d*, 12H, 4 CH_3-CH-, $J=6$ Hz), 1.96 and 2.13 (2*s*, $CH_3-C=CH-CHO$, *E*- and *Z*-isomers), 5.66 and 5.8 (2*bs*, olefinic protons), 9.81 and 9.85 (2*d*, 1H, $-CHO$, $J=7$ Hz, *E*- and *Z*-isomers). Phytenal (V) was converted into the enol acetate (VI and VII) by heating with isopropenyl acetate in the presence of catalytic amounts of *p*-toluenesulphonic acid and cupric acetate[6]. The enol acetates (VI and VII) were purified by passing through neutral alumina (grade II) and eluting exhaustively with pet. ether, yield 40%; PMR(CCl_4): δ 0.84 (*d*, 2H, 4 CH_3-CH-, $J=6$ Hz), 2.1 (*s*, 3H, $-OCOCH_3$), 4.87 (*bs*, $>C=CH_2$ of VII), 5.3 (*m*, C_4-H of VI), 5.96 (*d*, C_2-H, $J=12.5$ Hz), 6.34 (*d*, C_1-H, $J=12.5$ Hz). Stirring the enol acetates with sodium borohydride in ether-methanol (1:1) mixture according to literature procedure[7] gave a mixture of II, III and IV. These were separated by $AgNO_3/SiO_2$ (15%) chromatography and found to be identical [IR, NMR and GLC (column OV 101, temp. 170°)] with the alcohols obtained from the Prins reaction of I.

The mixture of alcohols, obtained from the Prins reaction of I, was condensed with equimolar amount of trimethylhydroquinone (VIII) by the procedure reported[8] for the condensation of chlorohydrin with trimethylhydroquinone. This gave α-tocopherol (IX; vitamin E) in 80% yield, identical in all respects with an authentic sample obtained from E Merck.

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A Convenient Synthesis of Polyhydroxylated Chalcones & Flavanones

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A convenient route has been described for the synthesis of polyhydroxy chalcones and flavanones involving demethylation of their methyl ethers with pyridinium bromide.

Polyhydroxylated chalcones and flavanones have been synthesised earlier as their methyl ethers followed by demethylation using reagents like hydroiodic acid and aluminium chloride in benzene. However treatment with such reagents leads to cleavage of the ring[1]. In the present note we describe the use of pyridinium bromide as a better demethylating agent.

Thus, fusion of 2′-hydroxy-4′-methoxychalcone[2] (**1**, 1 g) with pyridinium bromide (5 g) for 3 min followed by usual work-up led to 2′,4′-dihydroxychalcone (**1a**) in 80% yield. Similar reaction with 2′-hydroxy-4,4′-dimethoxy (**2**)[3]- and 2′-hydroxy-3, 4, 4′-trimethoxy-(**3**)[4]-chalcones, 7-methoxy[5]-(**4**)-, 7, 4′-dimethoxy[3]-(**5**)-, and 7, 3′, 4′-trimethoxy[6]-(**6**)-flavanone gave the corresponding hydroxy chalcones (**2a, 3a**) and flavanone (**4a, 5a** and **6a**). However dimethylation of 2′, 4′, 6′-trisubstituted chalcones such as 2′-hydroxy-4′, 6′-dimethoxy[7]-(**7**), 2′-hydroxy-4, 4′, 6′-trimethoxy[8]-(**8**)- and 2′-hydroxy-3, 4, 4′, 6′-tetramethoxy[9]-(**9**)-chalcone give directly 5, 7-dihydroxy-(**7a**)-, 4′, 5′, 7′-trihydroxy-(**8a**)-(naringenin), and 3′, 4′, 5, 7-tetrahydroxy-(**9a**)-flavanones in good yields (60-75%).

All the hydroxy chalcones and flavanones agreed in m.p. and PMR spectral data with literature values. Satisfactory analysis (C ± 0.25, H ± 0.13) were obtained for all the products.

The above results indicate that demethylation of chalcones having resorcinol moiety (2′,4′-substitution) in ring-A gives exclusively the hydroxychalcones. However, demethylation of chalcones having phloroglucinol moiety in ring-A (2′,4′,6′-trisubstitution), leads to exclusively flavanones.

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Syntheses of 7-Ethoxy-3,4-dimethylcoumarin & 5-Methoxy-4-methylcoumarin

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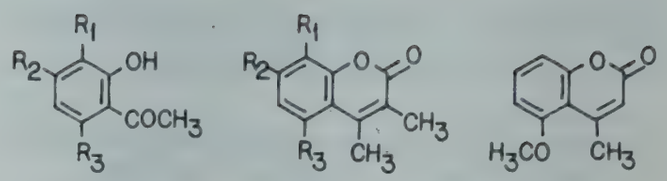
The structures proposed for two new naturally occurring coumarins as 7-ethoxy-3,4-dimethylcoumarin (**1a**) and 5-methoxy-4-methylcoumarin (**2**)(isolated from *Edgeworthia gardneri*) have been confirmed by their syntheses.

Two new coumarins isolated by Chatterjee *et al.*[1] from the stem bark of *Edgeworthia gardneri* were fully characterised on the basis of spectral evidences as 7-ethoxy-3,4-dimethyl coumarin (**1a**) and 5-methoxy-4-methylcoumarin (**2**). The present note describes convenient synthesis of **1a** and **2** in support of the assigned structures. During the course of the present work a convenient route has also been developed for the synthesis of 3,4-dimethylcoumarins such as **1b**, **1c** and **1d**.

7-Ethoxy-3,4-dimethylcoumarin (**1a**) was synthesised from 4-ethoxy-2-hydroxyacetophenone (**3a**) by condensing **3a** with (1-carbethoxyethylidene)triphenylphosphorane (**4a**)[2]. Spectral data and m.p. of **1a** were similar to those reported in literature. Further confirmation was provided by synthesising **1a** by Pechmann condensation of resorcinol with ethyl 2-methylacetoacetate followed by ethylation of formed 7-hydroxy-3,4-dimethylcoumarin (**1f**) with diethyl sulphate. Similarly 7-methoxy-3,4,8-trimethyl-(**1b**)-, 5-methoxy-3,4-dimethyl-(**1c**)-, 7-methoxy-3,4,5-trimethyl-(**1d**)-coumarins were obtained by Wittig reaction. For comparison purposes 5-methoxy-3,4,7-trimethylcoumarin (**1e**) was obtained by the Pechmann condensation of 1,3-dihydroxy-5-methylbenzene (orcinol) with ethyl 2-methylacetoacetate followed by methylation of the formed 5-hydroxy-3,4,7-trimethylcoumarin (**1h**) with dimethyl sulphate (Table 1). The formation of 5-hydroxy-3,4,7-trimethylcoumarin (**1h**) is in agreement with the earlier results[3] of Pechmann condensation of orcinol with ethyl acetoacetate which yielded 5-hydroxy-4,7-dimethylcoumarin.

5-Methoxy-4-methylcoumarin (**2**) was synthesised by the Wittig reaction of 2-hydroxy-6-methoxyacetophenone with ethoxycarbonylmethylenetriphenylphosphorane (**4b**). It may be appropriate to mention that **2** cannot be obtained by any other method.



(3a-d) (1a-h) (2)

3a, 1a $R_2=OCH_2CH_3$, $R_1=R_3=H$
3b, 1b $R_1=CH_3$, $R_2=OCH_3$, $R_3=H$, $Ph_3P=C(R)COOEt$
3c, 1c $R_3=OCH_3$, $R_1=R_2=H$
3d, 1d $R_2=OCH_3$, $R_3=CH_3$, $R_1=H$ (4a-b)
1e $R_3=OCH_3$, $R_2=CH_3$, $R_1=H$ 4a $R=CH_3$
1f $R_2=OH$, $R_1=R_3=H$ 4b $R=H$
1g $R_1=CH_3$, $R_2=OH$, $R_3=H$
1h $R_3=OH$, $R_2=CH_3$, $R_1=H$

Table 1—Characterization Data of 3,4-Dimethylcoumarins (**1b-1h**)

Compd	Yield (%)	m.p. °C	Mol. formula	Found/(Calc.) (%)	
				C	H
1b	68	177-78	$C_{13}H_{14}O_3$	71.6 (71.6)	6.4 (6.4)
1c	65	145-46	$C_{12}H_{12}O_3$	70.7 (70.6)	5.9 (5.9)
1d	60	153-54	$C_{13}H_{14}O_3$	71.6 (71.6)	6.4 (6.4)
1e	80	131-32	$C_{13}H_{14}O_3$	71.5 (71.6)	6.4 (6.4)
1f	87	238-40	$C_{11}H_{10}O_3$	69.4 (69.5)	5.3 (5.3)
1g	84	263-65	$C_{12}H_{12}O_3$	70.6 (70.6)	5.9 (5.9)
1h	76	251-52	$C_{12}H_{12}O_3$	70.5 (70.6)	5.9 (5.9)

7-Ethoxy-3,4-dimethylcoumarin (**1a**): *Typical procedure*

4-Ethoxy-2-hydroxyacetophenone (**3a**, 1g) and (1-carbethoxyethylidene)triphenylphosphorane (**4a**, 2.2 g) were powdered into an intimate mixture and heated on an oil-bath at 180° for (8-10 hr). The residue was subjected to column chromatography over silica gel. Elution with benzene-pet ether (1:1) gave the compound **1a** which crystallised from benzene-pet ether as colourless shining crystals (0.80 g, 66%), m.p. 121-22° (Found: C, 71.53; H, 6.45. $C_{13}H_{14}O_3$ requires C, 71.55; H,

6.42%); IR: 1700 (C=O); PMR($CDCl_3$): δ 1.44 (3H, *t*, *J*=7 Hz, OCH_2CH_3), 2.15 (3H, *s*, 3-CH_3), 2.35 (3H, *s*, 4-CH_3), 4.06 (2H, *q*, *J*=7 Hz, OCH_2CH_3), 6.75 (1H, *d*, *J*=2.5 Hz, H-8), 6.90 (1H, *dd*, *J*=9, 2.5 Hz, H-6), 7.55 (1H, *d*, *J*=9 Hz, H-5).

7-*Hydroxy*-3,4-*dimethylcoumarin* (**1f**):
Typical procedure

To a well colled mixture of resorcinol (2 g) and ethyl 2-methylacetoacetate (2.6 ml), sulphuric acid (5 ml) was added slowly with shaking at (0-5°). The viscous solution thus obtained was kept in referigerator for 24 hr and then poured into ice cold water (200 ml) with stirring. The separated product was filtered, washed with water and crystallised from ethanol as colourless crystal of **1f** (3.0 g, 87%), m.p. 238-40°.

5-*Methoxy*-4-*methylcoumarin* (**2**)

A mixture of 2-hydroxy-6-methoxyacetophenone (1 g) and ethoxycarbonylmethylenetriphenylphosphorane (**4b**, 2.5 g) was refluxed in anhydrous benzene (25 ml) for 36 hr. The solvent was distilled off and the residue subjected to column chromatography over silica gel. Elution with benzene-pet ether (1:1) gave **2**, which crystallised from benzene-pet ether as white shining crystals (0.82 g, 71%), m.p. 137-38° (Found: C, 69.50; H, 5.25. $C_{11}H_{10}O_3$ requires C, 69.47; H, 5.26%); IR: 1725 (≻C=O); PMR($CDCl_3$): δ 2.60 (3H, *s*, 4-CH_3), 3.90 (3H, *s*, OCH_3), 6.72 & 6.92 (each 1H, each *d*, *J*=8 Hz, H-6 & H-8), 7.45 (1H, *t*, *J*=8 Hz, H-7).

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New Route to Synthesis of Some 8-(Dihydrocinnamoyl)dihydrocoumarin Derivatives

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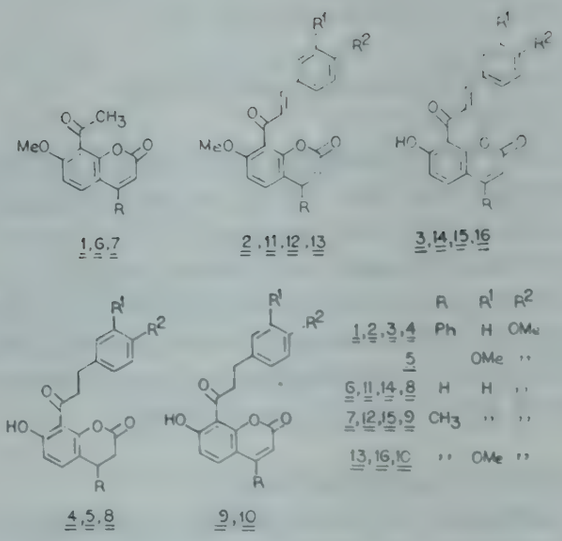
The synthesis of 8-dihydrocinnamoyldihydrocoumarin derivatives starting from 8-acetyl-7-methoxy substituted coumarins involves: (i) aldol condensation with substituted benzaldehydes; (ii) partial demethylation of condensation pfoduct ($AlCl_3$/acetonitrile); and (iii) catalytic hydrogenation of demethylated products over 10% Pd/C in ethyl acetate.

The present note deals with an unambiguous synthesis of 8-dihydrocinnamoyldihydrocoumarin derivatives. Our objective was to synthesise a naturally occurring 8-dihydrocinnamoyl-4-phenyldihydrocoumarin, which was isolated[1] from the fern *Pityrogramma calomelanos* and assigned the structure as 8- or 6-dihydrocinnamoyl-5,7-dihydroxy-4-phenyl-1-benzopyran-2(*H*)-one. However due to poor yields of 6- or 8-acetyl-5,7-dihydroxy-4-phenylcoumarin, it could not be synthesised.

8-Acetyl-7-methoxy-4-substituted-coumarins prepared by known procedures were condensed with different benzaldehydes to give 8-cinnamoyl-7-methoxy-4-substituted-coumarins, which on partial demethylation ($AlCl_3$/acetonitrile) and final hydrogenation (10% Pd/C) gave the required 8-dihydrocinnamoyl-dihydrocoumarins (Table 1).

Thus, condensation of 8-acetyl-7-methoxy-4-phenylcoumarin[2] (**1**) with 4-methoxybenzaldehyde gave 7-methoxy-8-(4′-methoxycinnamoyl)-4-phenylcoumarin (**2**), which on demethylation ($AlCl_3$/acetonitrile) afforded 7-hydroxy-8-(4′-methoxycinnamoyl)-4-phenylcoumarin (**3**). Hydrogenation of **3** over 10% Pd/C in ethyl acetate led to 3,4-dihydro-8-(α,β-dihydro-4′-methoxycinnamoyl)-7-hydroxy-4-phenylcoumarin (**5**).



Similar condensations of 8-acetyl-7-methoxycoumarin (**6**) and 8-acetyl-7-methoxy-4-methylcoumarin (**7**) with 4-methoxybenzaldehyde led to the corresponding 3,4-dihydro-8-(α,β-dihydro-4′-methoxycinnamoyl)-7-hydroxycoumarin (**8**) and 8-(α,β-dihydro-4′-methoxycinnamoyl)-7-hydroxy-4-methylcoumarin (**9**) after demethylation of the formed **11** and **12** respectively. Condensation of **6** with 3,4-dimethoxybenzaldehyde afforded 8-(α,β-dihydro-3′,4′-dimethoxycinnamoyl)-7-hydroxy-4-methylcoumarin (**10**) after demethylation of formed **13**. Hydrogenation of **8-10** afforded **14-16** respectively.

Spectral data (IR, PMR) of compounds synthesised were compatible with the proposed structures.

Table 1—Characterization Data of (**2-5**, **8-16**)

Compd.	Yield (%)	m.p. (°C)	Mol. formula*
2	85	120-21	$C_{26}H_{20}O_5$
3	70	198-99	$C_{25}H_{18}O_5$
4	65	126-27	$C_{25}H_{22}O_5$
5	65	120-21	$C_{26}H_{24}O_6$
8	70	172-73	$C_{19}H_{18}O_5$
9	60	101-2	$C_{20}H_{18}O_5$
10	65	157-58	$C_{21}H_{20}O_6$
11	80	152-53	$C_{20}H_{16}O_5$
12	65	190-91	$C_{21}H_{18}O_5$
13	70	200-01	$C_{22}H_{20}O_6$
14	75	187-88	$C_{19}H_{14}O_5$
15	65	182-83	$C_{20}H_{16}O_5$
16	70	157-58	$C_{21}H_{18}O_6$

*All the compounds gave C, H and N analyses well within the acceptable limits (±0.4%).

7-Methoxy-8-(4′-methoxycinnamoyl)-4-phenylcoumarin (**2**): *General procedure*

Compound **1** (1 g) in ethyl alcohol and 4-methoxybenzaldehyde (0.50 ml) in the presence of potassium hydroxide were mixed and stirred for 6 hr at 35-40°. Usual work-up of the rection mixture gave a solid which was passed through a column of silica gel. Elution with benzene-pet. ether (3:2) gave **2** as yellow needles (1 g), m.p. 120-21°; IR(KBr): 1730 cm^{-1} (C=O of coumarin moiety);

PMR($CDCl_3$): δ 3.88 and 3.91 (each *s*, each 3H, $2\times OCH_3$), 6.23 (*s*, 1H, H-3), 6.89 (*d*, *J*=9 Hz, 1H, H-6), 7.01 (*d*, *J*=9 Hz, H-3′ and H-5′), 7.29-7.55 (*m*, 9H, H-α, H-2′, H-5, H-6′ and Ph), 7-7.1 (*d*, *J*=17 Hz, 1H, H-β).

7-Hydroxy-8-(4′-methoxycinnamoyl)-4-phenylcoumarin (**3**): *General procedure*

A solution of **2** (1 g) in acetonitrile (10 ml) was added with stirring to a solution of aluminium chloride (0.5 g) in acetonitrile (30 ml) during 30 min at room temperature. Stirring was continued for further 8 hr at 40-45°, the reaction mixture poured into crushed ice and 5*N* hydrochloric acid (25 ml) added. It was extracted with ether, ether layer washed with aq. sodium hydroxide (10%), dried (Na_2SO_4) and solvent distilled off to give a solid which was passed through a column of silica gel. Elution with benzene gave yellow shining crystals of **3** (0.70 g), m.p. 198-99°; PMR($CDCl_3$): δ 3.90 (*s*, 3H, OCH_3), 6.30 (*s*, 1H, H-3), 6.90 (*d*, *J*=9 Hz, 1H, H-6), 7.03 (*d*, *J*=9 Hz, 2H, H-3′ and H-5′), 7.30-7.80 (*m*, 11H, H−α, H−β, H-2′, H-5, H-6′ and Ph), 14.22 (*s*, 1H, exchangeable with D_2O, 7-OH).

3,4-Dihydro-8-(α,β-dihydro-4′-methoxycinnamoyl)-7-hydroxy-4-phenylcoumarin (**4**): *General procedure*

A solution of **3** (0.5 g) in anhyd ethyl acetate (25 ml) was hydrogenated for 6 hr over Pd/C (10%, 0.4 g). Usual work-up gave a solid which was crystallised from benzene-pet. ether to give **4** as colourless crystals (0.26 g), m.p. 126-27°; PMR($CDCl_3$): δ 2.80-3.15 (*m*, 4H, H−α, H−α′, and H−3), 3.51 (*t*, *J*=7 Hz, H−β & H−β′), 3.79 (*s*, 3H, OCH_3), 4.29 (*t*, *J*=7 Hz, 1H, H−4), 6.70-7.50 (*m*, 11H, H−2′, H−3′, H−5, H−5′, H−6, H−6′ and Ph), 14.31 (*s*, 1H, exchangeable with D_2O; 7−OH).

3,4-Dihydro-8-(α,β-dihydro-4′-methoxycinnamoyl)-7-hydroxy-4-phenylcoumarin (**4**): *General procedure*

A solution of **3** (0.5 g) in anhyd ethyl acetate (25 ml) was hydrogenated for 6 hr over Pd/C (10%, 0.4 g). Usual work-up gave a solid which was crystallised from benzene-pet ether to give **4** as colourless crystals (0.26 g), m.p. 126-27°; PMR($CDCl_3$): δ 2.80-3.15 (*m*, 4H, H−α, H−α′ and H−3), 3.51 (*t*, *J*=7 Hz, H−β & H−β′), 3.79 (*s*, 3H, OCH_3), 4.29 (*t*, *J*=7 Hz, 1H, H-4), 6.70-7.50 (*m*, 11H, H-2′, H-3′, H-5, H-5′, H-5′, H-6, H-6′ and Ph), 14.31 (*s*, 1H, exchangeable with D_2O; 7−OH).

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2-Phenyl-6-cinnamoylchromans as Synthones for 2-Amino- & 2-Mercapto-4,5-substituted-thiazoles

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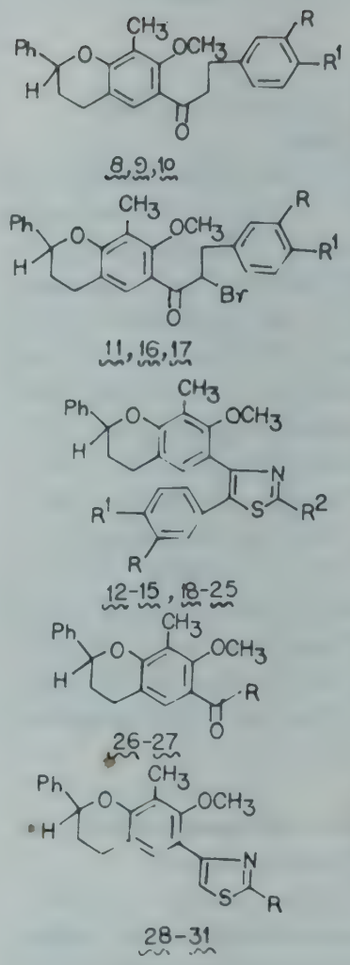
Some 2-amino- and 2-mercapto-4-aryl-5-arylmethylthiazoles (**12**, **18** & **19** and **14**, **22**, **23**) have been synthesised by the condensation of α-bromo-α,β-dihydrocinnamoyl-3,4-dihydro-2-phenyl-2*H*-1-benzopyrans (**11**, **16** and **17**) with thiourea and ammonium dithiocarbonate, respectively. The required bromo derivatives have been prepared by the bromination of dihydrocinnamoyl derivatives (**8**, **9** and **10**) which in turn have been obtained by the hydrogenation of cinnamoyl derivatives (**2**, **3** and **4**) followed by methyltion. None of the compounds shows any noteworthy antifungal and antibacterial activities.

Thiazoles are known to possess CNS stimulant[1], antitubercular[2,3], antibacterial[4,5], fugicidal[6-9], anthelmintic[10], hypotensive and hypothermic[11] activities. 2-Phenyl-2*H*-1-benzopyrans (flavans) are also reported for their biological activity[12]. Therefore, we have synthesised some 3,4-dihydrobenzopyranyl-5-arylmethylthiazoles from 2-phenyl-6-cinnamoylchromans. Condensation of 6-acetyl-3,4-dihydro-7-hydroxy-8-methyl-2-phenyl-2*H*-1-benzopyran (2-phenylchroman derivative) (**1**)[13] with anisaldehyde, veratraldehyde and tolualdehyde gave the 6-cinnamoyl derivatives **2**, **3** and **4**, respectively which on hydrogenation with Pd/C gave the corresponding dihydrocinnamoyl derivatives (**5**, **6** and **7**). The latter compounds (**5**, **6**, **7**) on methylation with dimethyl sulphate in acetone-potassium carbonate gave the corresponding 7-methoxydihydrocinnamoyl derivatives (**8**, **9** and **10**).

As a representative case, the compound **8** on bromination (bromine in CCl_4) gave a product which responded positively to DNP test. On the basis of its spectral data (see experimental) its structure was established as 3,4-dihydro-6-(α-bromo-α,β-dihydro-4′-methoxycinnamoyl)-7-methoxy-8-methyl-2-phenyl-2*H*-1-benzopyran (**11**). **11** on condensation with thiourea or ammonium dithiocarbamate in abs. ethanol afforded 2-amino-4-(3′,4′-dihydro-7′-methoxy-8′-methyl-2′-phenyl-2′*H*-1′-benzopyran-6′-yl)-5-(4″-methoxyphenylmethyl)thiazole (**12**) or 4-(3′,4′-dihydro-7′-methoxy-8′-methyl-2′-phenyl-2′*H*-1′-benzopyran-6′-yl)-5-(4″-methoxyphenylmethyl)-2-mercaptothiazole (**14**) respectively. The presence of amino group in **12** was confirmed by PMR data of its acetate



	R	R^1	R^2
8,11	H	OCH_3	–
9,16	OCH_3	,,	–
10,17	H	CH_3	–
12	,,	OCH_3	NH_2
13	,,	,,	$NHCOCH_3$
14	,,	,,	SH
15	,,	,,	SMe
18	OCH_3	,,	NH_2
19	H	CH_3	,,
20	OCH_3	OCH_3	$NHCOCH_3$
21	H	CH_3	,,
22	OCH_3	OCH_3	SH
23	H	CH_3	,,
24	OCH_3	OCH_3	SMe
25	H	CH_3	,,
26	CH_3	–	–
27	CH_2Br	–	–
28	NH_2	–	–
29	SH	–	–
30	$NHCOCH_3$	–	–
31	SMe	–	–

(**13**) prepared by treatment with acetic anhydride in benzene. Similarly, the presence of mercapto group in **14** was confirmed by its methyl derivative (**15**) prepared by reacting with dimethyl sulphate in acetone containing K_2CO_3.

Similarly compounds **9** and **10** on bromination gave the bromo derivatives **16** and **17** respectively which on condensation with thiourea or ammonium dithiocarbamate yielded the corresponding 2-aminothiazoles (**18** and **19**) or 2-mercaptothiazoles (**22** and **23**).

The starting compound **1** was also methylated to give **26** and treated with bromine to give the α-bromo compound (**27**) which on condensation with thiourea and ammonium dithiocarbamate gave the corresponding 2-aminothiazole (**26**) and 2-mercaptothiazole (**29**).

Structures of all the compounds prepared were in agreement with their PMR spectral data and elemental analyses.

Biological activity

Compounds **12-15**, **18-25** and **28-31** were tested against the fungi *Aspergillus fumigatus* and *A. niger* at

10 μg/ml and 20 μg/ml concentrations. Among these compounds, **28** showed maximum inhibition at 10 μg/ml against *A. niger* while other compounds showed moderate activity.

The compounds were also tested for their *in vitro* antibacterial activity against *Pseudomonas pyocyanea* and *Erscherichia coli*. Compound **13** showed maximum activity at 20 μg/ml concentration while **25** exhibited good activity at 10 μg/ml concentration against *E. coli*.

6-(α-Bromo-α,β-dihydro-4′-methoxycinnamoyl)-3,4-dihydro-7-methoxy-8-methyl-2-phenyl-2H-1-benzopyran (**11**): *Typical procedure*

Bromine (0.48 g as 10% solution in carbon tetrachloride) was added dropwise to a solutions of 3,4-dihydro-6-(α,β-dihydro-4′-methoxycinnamoyl)-7-methoxy-8-methyl-2-phenyl-2*H*-1-benzopyran (**8**; 1 g)[13] with stirring at 30-35° during 40 min. The solvent was distilled off and the residue purified by passing through a column of silica gel using benzene-pet. ether (1:19) as eluant when **11** was obtained as yellow oil (1.2 g, 80%); PMR($CDCl_3$): δ 2.01 (*m*, 2H, H-3), 2.15 (*s*, 3H, CH_3), 2.75 (*m*, 2H, H-4), 3.10-3.30 (*m*, 2H, H-β), 3.40 (*s*, 3H, 7-OCH_3), 3.80 (*s*, 3H, 4′-OCH_3), 5.10 (*dd*, 1H, H-2), 5.65 (*t*, *J* = 7 Hz, 1H, H-α), 6.90 (*d*, *J* = 9 Hz, 2H, H-3′ and H-5′), 7.11 (*d*, *J* = 9 Hz, 2H, H-2′ and H-6′), 7.25 (*s*, 1H, H-5), 7.40 (*s*, 5H, Ph).

Following compounds were similarly obtained:
16: Oil, yield 85%.
17: Oil, yield 85%.
27: Oil, yield 90%.

2-Amino-4-(3′,4′-dihydro-7′-methoxy-8′-methyl-2′-phenyl-2′H-1′-benzopyran-6′-yl)-5-(4″-methoxyphenylmethyl)thiazole (**12**): *Typical procedure*

A solution of **11** (1 g) in abs. ethanol (25 ml) was refluxed for 4 hr with thiourea (0.16 g) and the solvent distilled off. The solid obtained after the addition of crushed ice and liquor ammonia was filtered, washed with water, dried and crystallised from benzene to give **12** as pale yellow crystals (0.7 g, 70%), m.p. 210-11°; PMR($CDCl_3$ + 1 drop TFA): δ 2.10 (*m*, 2H, H-3′), 2.20 (*s*, 3H, 8′-CH_3), 2.80 (*m*, 2H, H-4′), 3.65 (*s*, 3H, 7′-OCH_3), 3.85 (*s*, 3H, 4″-OCH_3), 3.99 (*s*, 2H, CH_2), 5.11 (*dd*, 1H, H-2′), 6.90 (*d*, *J* = 9 Hz, 2H, H-3″ and H-5″), 7.11 (*d*, *J* = 9 Hz, 2H, H-2″ and H-6″), 7.35 (*s*, 6H, H-5′ and Ph); acetyl derivative (**13**): yield 80%, m.p. 135-37°.

Following compounds were similarly obtained.
18: m.p. 207-8°, yield 75%; acetyl derivative (**20**): m.p. 173-74°, yield 75%.
19: m.p. 197-98°, yield 70%; acetyl derivative (**21**): m.p. 160-61°, yield 65%.
28: m.p. 99-101°, yield 55%; acetyl derivative (**30**): m.p. 188-90°, yield 85%.

2-Mercapto-4-(3′,4′-dihydro-7′-methoxy-8′-methyl-2′-phenyl-2′H-1′-benzopyran-6′-yl)-5-(4″-methoxyphenylmethyl)thiazole (**14**): *Typical procedure*

Ammonium dithiocarbamate (0.25 g) was added to a solution of **11** (1 g) in abs. ethanol (25 ml) with stirring, the mixture refluxed for 1 hr and the solvent distilled off. The residue was refluxed with benzene (10 ml), filtered and the filtrate cooled to afford **14** as colourless crystals (0.75 g, 60%), m.p. 168-70°; PMR($CDCl_3$): δ 1.65 (*s*, 1H, SH, exchangeable with D_2O), 2.05 (*m*, 2H, H-3′), 2.18 (*s*, 3H, 8′-CH_3), 2.83 (*m*, 2H, H-4′), 3.59 (*s*, 3H, 7′-OCH_3), 3.75 (*s*, 3H, 4″-OCH_3), 3.82 (*s*, 2H, CH_2), 5.12 (*dd*, 1H, H-2′), 6.79 (*d*, 2H, H-3″ and H-5″), 7.1 (*d*, 2H, H-2″ and H-6″), 7.3 (*s*, 1H, H-5′), 7.4 (*s*, 5H, C_6H_5).

Following compounds were similarly obtained:
22: m.p. 144-45°, yield 60%; methyl derivative (**24**): oil, yield 55%.
23: m.p. 204-5°, yield 50%; methyl derivative (**25**): oil, yield 55%.
29: m.p. 122-23°, yield 50%; methyl derivative (**31**): m.p. 105-6°, yield 45%.

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Thermolytic Reaction of Chalcone with Sulphur: Formation of Organo-Sulphur Compounds

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Transoid and *cisoid*-2-benzoyl-3-thiobenzoyl-1,4-diphenylbutadienes (I and II) have been identified in the thermolytic reaction of chalcone with sulphur, at 250°C under N_2-atmosphere.

In previous communications from our laboratory[1-3], the formation of the following compounds was reported in the thermolytic reaction of chalcone with sulphur: dihydrochalcone, 2,4,5-triphenylthiophene, *trans, trans, trans*-2-benzoyl-2-benzoylthio-1,3-diphenylcyclobutane and 4,5-dibenzoyl-3,6-diphenyl-1,2-dithi-4-ene. Herein we report the formation of two more compounds, *transoid*- and *cisoid*-2-benzoyl-3-thiobenzoyl-1,4-diphenylbutadienes in the reaction of chalcone with sulphur carried out at 250° for 4 hr under N_2-atmosphere.

In a typical reaction chalcone (3.12 g) and sulphur (0.96 g) were heated together at 250° (oil-bath) for 4 hr under N_2-atmosphere. After the evolution of H_2S had ceased, the reaction mixture was chromatographed (SiO_2) and elution of the column with light petroleum ether furnished a mixture of two compounds (A) and (B). This mixture was subsequently resolved by preparative layer chromatography over silica gel using the same solvent systems; yield: (A), 27 mg; and (B) 25 mg.

Compound (A) recrystallized from acetone in yellow crystals, m.p. 178-80° (uncorr). In the mass spectrum the M^+ appeared at *m/z* 430, while the intensity of (M+2) ion peak was indicative of the presence of one sulphur atom in the molecule, in conformity with the molecular formula, $C_{30}H_{22}OS$. The presence of benzoyl and thiobenzoyl groups in (A) was indicated by the mass peaks at *m/z* 105 and 121 respectively. Its molecular weight (430) suggested that two chalcone molecules have combined with attendant loss of two hydrogen atoms and replacement of one of the carbonyl oxygen by sulphur.

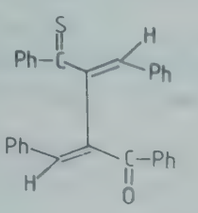
The IR spectrum of (A) in KBr exhibited bands at 1590, 1490, 770, 755, 700 cm^{-1}, which suggested the presence of monosubstituted phenyl. The other bands were present at 1660 (sh) and 1590 ($\nu_{C=C}$ of a diene); 980 (δCH transoid diene)[4], 1685 ($\nu_{C=O}$, conjugated) and 1170 cm^{-1} ($\nu C=S$)[4].

The UV spectrum of (A), in CH_3CN displayed two prominent peaks at 250 and 340 nm. The primary band at 250 nm was presumably due to benzene ring (the *K*-band of benzene ring at 204 nm has apparently shifted to 250 nm due to substitution). The secondary band at 340 nm was due to extended conjugation (transoid) in the molecule.

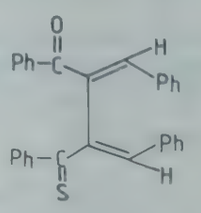
The 60 MHz PMR spectrum of (A) in $CDCl_3$ displayed a sharp one-proton singlet at δ 5.25 assignable to the vinylic proton (adjacent to the carbon bearing the thiocarbonyl group). The other one-proton singlet at δ 6.7 was assigned to another vinylic proton adjacent to the carbon bearing the benzoyl group. A complex multiplet, centred at δ 7.55, integrated for 20 aromatic protons.

Based on the above spectral data, compound (A) has been identified as the *transoid*-2-benzoyl-3-thiobenzoyl-1,4-diphenylbutadiene.

Compound (B) crystallized from acetone in yellow needles m.p. 136-37° (uncorr). The IR spectrum of (B) in KBr exhibited bands at 3050, 3020, 1590, 1480, 770, 760, 690 (monosubstituted phenyl), 1680 ($\nu_{C=O}$, conjugated), 1170 ($\nu_{C=S}$)[4], 1660 (sh), 1590 ($\nu_{C=C}$ of a diene), and 735, 730, 725, 720 cm^{-1} (*cisoid* type of diene)[4]. The UV spectrum of (B) in CH_3CN displayed two prominent bands at 250 (K-band of benzene) and 390 nm (extended conjugation, *cisoid* type). The mass spectrum of (B) showed M^+ at *m/z* 430 and the fragmentation paatern was similar to that of compound (A). However, the intensities of the peaks were greater in (B) as compared to that in (A). This observation leads to the conclusion that the two compounds are isomers. The 60 MHz PMR spectrum of (B) in $CDCl_3$ exhibited differences in the location of the downfield vinylic proton (δ 6.9) as well as in the splitting pattern of the 20 aromatic protons. Here the complex multiplet was centred at δ 7.4.



(I)



(II)

Based on the spectral data structrure (II) was assigned to compound (B).

We wish to thank Dr K P Madhusudanan and Mr R K Singh (RSIC, Lucknow) for carrying out the mass spectral analysis of the compounds.

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A Short Route for the Synthesis of 2-Substituted 5,5-Ethylenedioxypentan-1-als†

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A short synthesis of 5,5-ethylenedioxypentanol (**1**) has been described via an alkylation-reduction sequence.

The title compounds **1a** and **1c-e** were used as intermediates in the synthesis of samandarine analogues by Reddy *et al.*[1b] and of (±)-khusitene (from **1c**) by Vig *et al.*[2] Recently Fujisawa *et al.*[3] have reported one-pot conversion of a carboxylic acid group into an aldehyde moiety in the presence of several other easily reduceable functional groups. We describe herein a modified route for the synthesis of the title compounds.

Freshly recrystallised and dried 3-phenylpropionic acid was treated with N,N-dimethylchloromethyleniminium chloride tri-*t*-butoxyaluminium hydride according to the procedure of Fujisawa *et al.*[3] Decomposition of the lithium complex was then attempted using mild reagents. After a few trials, saturated solution of potassium sodium tartrate was found to give best results. Usual work-up gave 3-phenylpropanal in 75% yield. Since potassium sodium tartrate solution does not cleave acetal protection[1b], this procedure was extended for the synthesis of the title compounds.

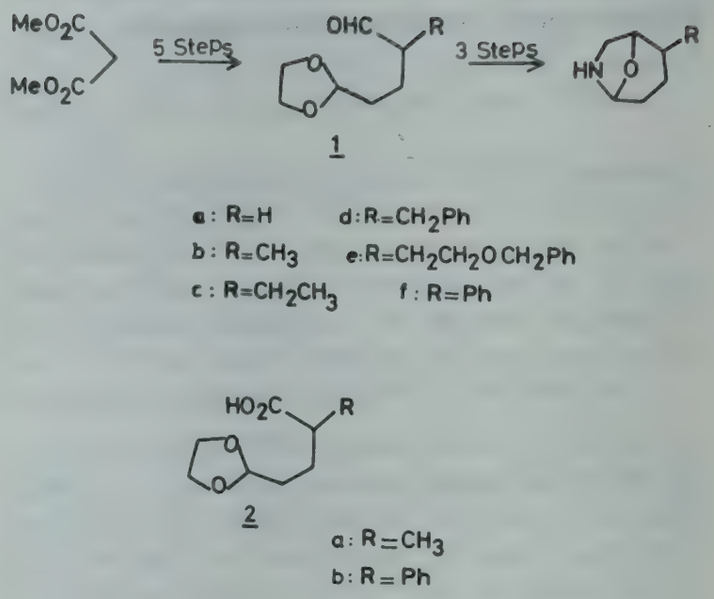
The required starting acids **2a** and **2b** were prepared by lithio-dianion alkylation of the corresponding carboxylic acid 2-(2-bromoethyl)-1,3-dioxalane according to the procedure of Joshi *et al.*[4] (yields 70%). Direct reduction of these acids to the corresponding aldehydes above for 3-phenylpropionic acid (yields 60-63%).

The short route reported in this note for the synthesis of 2-substituted 5,5-ethylenedioxypentan-1-als (**1**) has the following advantages over the earlier routes:

(i) The number of steps have been reduced from five to two.

(ii) The overall yields have been improved from 12% to 45%.

(iii) The new permits introduction of aryl substituents, a facility not available via malonic ester route reported earlier[1b,2].



The following represents the typical exerpimental procedure:

Compound **1b**

Oxalyl chloride (2.6 ml) was added to a solution of N,N-dimethylformamide (0.8 ml, 10.5 mmol) in dichloromethane (16 ml) at 0°, the solution stirred for 1 hr, solvent removed under reduced pressure and to the residual white powder were added acetonitrile (16 ml) and THF (26 ml). Thereafter, a solution of 5,5′-ethylenedioxy-2-methylpentaoic acid (1.82 g, 10.5 mmol) in pyridine (0.9 ml) − THF (16 ml) was added to the above mixture at −30°. This reaction mixture was cooled at −78° and to it were added a suspension of 10 mol% of copper(I) iodide in THF and a solution of $LiAlH(OBu^t)_3$ (5.32 g, 21 mmol) in THF (16 ml). After stirring for 10 min, the reaction was quenched by the addition of sodium potassium tartrate solution. The organic layer was extracted with ether (6 × 30 ml), washed with aq. $NaHCO_3$ solution, dried ($MgSO_4$), solvent removed on a rotary evaporator and the residue distilled under reduced pressure to get **1b**, yield 1.07 g (65%), b.p. 85-90°/10-11 mm (bath temperature) (Found: C, 60.7; H, 8.8. $C_8H_{14}O_3$ requires C, 60.8; H, 8.9%).

Compound **1f** (R = Ph) was prepared in a similar way, yield 61%, b.p. 120-125°/0.3 mm (bath temperature) (Found: C, 70.6; H, 7.2. $C_{13}H_{16}O_3$ requires C, 70.9; H, 7.3%).

†Synthesis of bicyclic oxazolidines: Part III. For Parts I and II of this series see refs 1a and 1b respectively.

Two of the authors (RV and BV) are thankful to CSIR, New Delhi for the award of junior research fellowships.

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Lactam Acetals: Part XV – Reaction with Grignard Reagents

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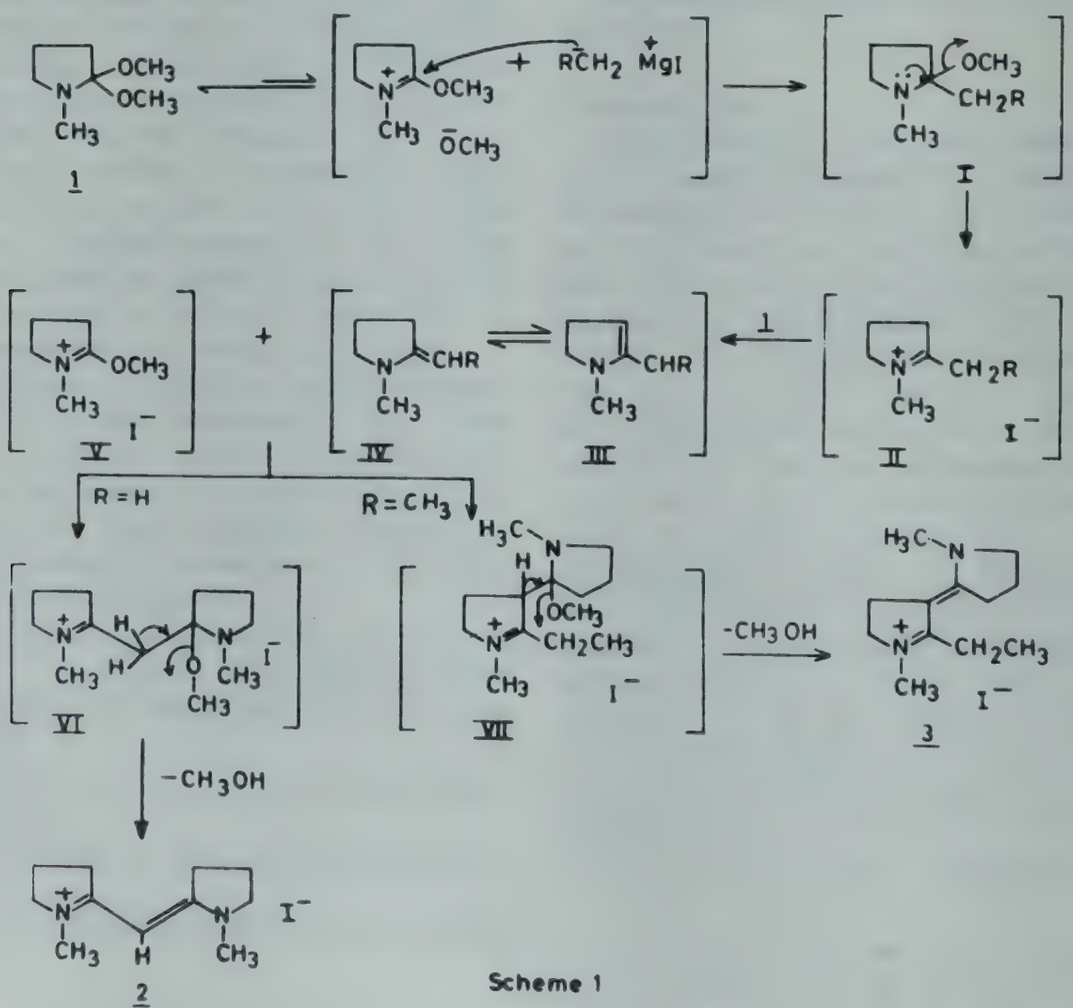
Reaction of 2,2-dimethoxy-1-methylpyrrolidine (**1**) with methyl- and ethyl-magnesium iodides results in the formation of 2-(1-methyl-2-pyrrolidinylidene)methylene-1-methyl-Δ^1-pyrrolinium iodide (**2**) and 2-ethyl-3-(1-methyl-2-pyrrolidinylidene)-1-methyl-Δ^1-pyrrolinium iodide (**3**) respectively.

In a continuing study of the reactions of lactam and amide acetals with nucleophiles[1-3], the reaction of 2,2-dimethoxy-1-methylpyrrolidine (**1**) with Grignard reagents, namely methyl- and ethyl-magnesium iodides has been investigated and the results are reported in this note.

Reaction of **1** with methylmagnesium iodide resulted in the formation of **2**, m.p. 143°, yield 30%; IR(KBr): 2900, 1580, 1300, 1260, 1100, 820, 800 cm^{-1}; PMR ($CDCl_3$): δ 2.22 (quintet, 4H, 4-CH_2, 4′-CH_2), 3.21 (*s*, 6H, 2×N−CH_3), 3.81 (*t*, 4H, 5-CH_2, 5′-CH_2), 5.00 (*s*, 1H, olefinic-H). The triplets of 3-CH_2 and 3′-CH_2 merged with the N−CH_3 signal, centred at 3.21.

However, ethylmagnesium iodide reacted with **1** to give the angular dimer **3**, m.p. 223-25°, yield 29.7%; IR (KBr): 3250, 3100, 2920, 2850, 1640, 1590, 930, 900, 700 cm^{-1}; PMR ($CDCl_3$): δ 1.19 (*t*, 3H, 2β-CH_3), 2.04 (quintet, 2H, 4′-CH_2), 2.80-3.35 (*m*, 4H, 4-CH_2 and 3-CH_2), 3.12 (*s*, 3H, N′-CH_3), 3.16 (*s*, 3H, N−CH_3) and 3.34-3.81 (*m*, 6H, 5-CH_2, 5′-CH_2 and 2α-CH_2). Spin decoupling experiments and NOE studies of **3** (10% deoxygenated solution in $CDCl_3$) revealed the following interesting features.



Scheme 1

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Irradiation of 2β-CH_3 (at δ 1.19) resulted in the simplification of the spectrum in the region of 3.34-3.81 with the appearance of a singlet due to 2α-CH_2 at 3.67. Simplification of the spectrum owing to irradiation of 4′-CH_2 helped in the assignments of the signals due to 3′-CH_2 and 5′-CH_2 appearing at δ 3.00 and 3.43 respectively. Similarly, irradiation of signal at δ 3.00 or 3.43 converted the qunitet at 2.04, due to 4′-CH_2, into a triplet. In the NOE study, irradiation of 3′-CH_2 signal resulted in 20% increase in the integral value for 2β-CH_3 while there was no appreciable enhancement in this signal on irradiation of N′-CH_3 signal. These results thus led to the assignment of *E*-stereochemistry to **3**.

Towards rationalisation for the formation of **2** and **3**, we believe that the first step involves the nucleophilic attack of Grignard reagent at 2-position of the lactam acetal. The resulting intermediate (I) may form an enamine with exocyclic (III) or endocyclic (IV) double bond via intermediate (II), which would further react with immonium specie V to form dimer VI or VII. In the case of methylmagnesium iodide, the formation of the linear dimer VI is favoured which gives rise to **2** by the loss of a molecule of CH_3OH, while ethylmagnesium iodide gives rise to the angular isomer (**3**) via the intermediate VII (see Scheme 1).

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Photoisomerisation of 4-Benzalamino-3-methyl-5-styrylisoxazole

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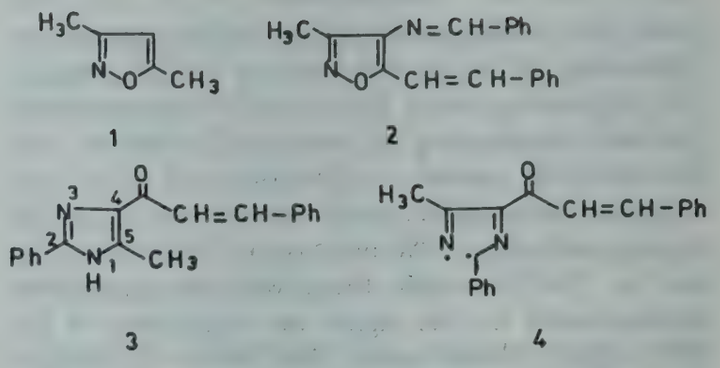
The attempted photochemical electrocyclic ring closure of 4-benzalamino-3-methyl-5-styrylisoxazole (**2**) leads to the imidazole (**3**) on account of isoxazole ring cleavage. The structure of **3** is supported by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data.

A few reactions on 4-benzalamino-3-methyl-5-styrylisoxazoles, viz. 1,3-dipolar cycloaddition of nitrile oxides and [2 + 2] photocycloaddition of tetracyanoethylene, which resulted in oxadiazolines[1] and cyclobutanes[2] respectively, have been reported from our laboratory. We have looked at the styryl and the azomethine functionalities in the aforementioned isoxazole as a 1,3,5-azahexatriene system. In view of this and in continuation of our interest on the photochemistry of isoxazoles, the results of attempted photochemical electrocyclic ring closure of 4-benzalamino-5-styrylisoxazole are reported in this note.

4-Benzalamino-3-methyl-5-styrylisoxazole (**2**)[1] was prepared from 3,5-dimethylisoxazole (**1**) by a sequence of reactions involving nitration, regiospecific styrylation, nitro group reduction and schiff base formation.

The photolysis of **2** was carried out by exposing its dilute solution in dry benzene to 2537 Å radiation under a nitrogen blanket for 8 hr. Removal of solvent at reduced pressure, followed by chromatography of the residue over a column of alumina gave a TLC pure product. The salient spectral data of the product were the appearance of a signal at δ 176 in its ^{13}C NMR spectrum (conjugated carbonyl), and a narrow *AB* quartet in the region δ 7.81-7.88, (a *trans* styryl double bond) and a broad and D_2O washable peak at 9.4 (> NH) in its ^{1}H NMR spectrum. The molecular ion peak was observed at *m/z* 288. The IR spectrum exhibited prominent bands at 1660 and 3400 cm^{-1} due to conjugated carbonyl and NH respectively. The spectral data of the product showed that it was not formed from **2** by electrocyclic ring closure. An over-view of the literature on isoxazole stability under a variety of conditions[3] revealed that the N – O bond is very readily cleaved. Based on this observation we have identified the product as 4(5)-cinnamoyl-5(4)-methyl-2-phenylimidazole (**3**), which turned out to be identical with



1 2 3 4

Chart 1

the product (m.p. 212°) obtained by Desimoni *et al.*[4] by thermal rearrangement of **2** in inert solvents. The product **3** arises by ring cleavage of **2** followed by rearrangement and cyclization of **4** (chart 1).

Irradiation of 4-benzalamino-3-methyl-5-styryl-isoxazole

A solution of **2** (0.001 mol) in dry benzene (185 ml) was irradiated at 2537 Å for 8 hr in a quartz tube after degassing. Removal of the solvent under reduced pressure gave a sticky material which was chromatographed over neutral alumina. The unreacted **2** eluted first, and the imidazole (**3**) was eluted with 6:4 benzene-ethyl acetate mixture. The product (**3**) was further processed by trituration with pet. ether, m.p. 212° (ref[4]. 211°) (Found: C, 79.1; H, 5.6; N, 9.7. $C_{19}H_{16}N_2O$ requires C, 79.2; H, 5.6; N, 9.7%); PMR($CDCl_3$): δ 2.6 (*s*, 3H, CH_3), 7.0-7.7 (*m*, 10H, Ar – H), 7.81-7.88 (*ABq*, 2H, – CH = CH –, *J* = 15 Hz), 9.4 (*bs*, 1H, NH); ^{13}C NMR($CDCl_3$): δ 9.0 (CH_3), 112 (– CO – *C*H =), 128 (= CH – Ph), 129 (C-4), 135 (C-2), 152 (C-5), 176 (– CO –), 118-130 (Ar – C).

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Hydrazinolysis of Unsaturated Azlactones & Cyclisation of the Acrylic Acid Hydrazides into Tetrahydrotriazine, Pyrazolidine & Imidazoline Derivatives

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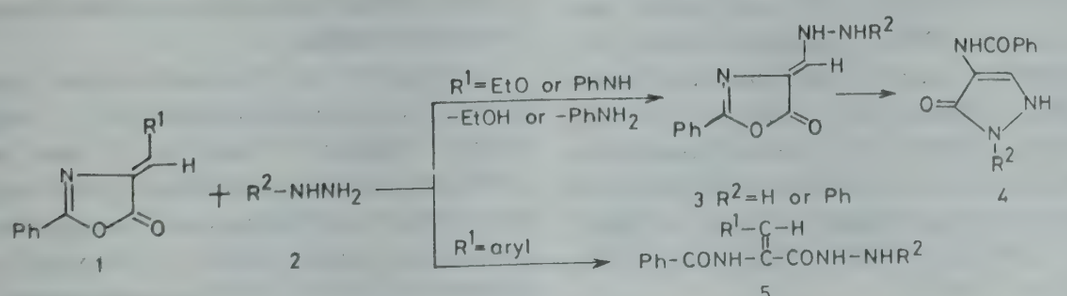
Hydrazinolysis of 4-arylmethylene-2-oxazolin-5-ones (**1**) gives the corresponding acrylic acid hydrazides (**5**) among which the N-substituted hydrazides (**5**; $R^2 = H$) afford 5-arylmethylene-3-phenyl-1,2,5,6-tetrahydro-1,2,4-triazin-6-ones (**6**) and 5-aryl-4-benzoylaminopyrazolidin-3-ones (**8**) on heating with aq. sodium hydroxide and with excess of hydrazine hydrate, respectively. However, the N-phenylhydrazides (**5**; $R^2 = Ph$) give the corresponding 1-anilino-4-arylmethylene-2-phenyl-2-imidazolin-5-ones (**9**) by simple heating. All the steps can be carried out in the same flask.

Various workers have studied the hydrazinolysis of azlactones and reported different results[1-4]. In connection with the aminolysis of azlactones (**1**)[5], hydrazinolysis of **1** and cyclisation of the resultant hydrazides (**5**) have now been reinvestigated. Whereas cleavage of the 1,5-bond of **1** occurred in all the cases, the reaction sequence depended on the nature of the reactants. In agreement with the earlier results[1], the azlactone **1** ($R^1 = EtO$) underwent substitution of the ethoxy group followed by rearrangement to **4**. Similar transformation was observed[6] in the case of **1b** ($R^1 = PhNH$). On the other hand, 4-arylmethylene-2-oxazolin-5-ones (**1c-e**; $R^1 =$ aryl) gave hydrazides (**5a-f**) which underwent cyclisation to different products depending on the type of substituents and the reaction conditions.

It was found that N-unsubstituted 2-benzoylamino-3-arylic acid hydrazides (**5**, $R^2 = H$) afforded triazinones (**6**) or heating with aq. sodium hydroxide for a few minutes. On the other hand, **5a** and **5e** gave pyrazolidinones (**8**) when heated with excess of hydrazine hydrate in ethanol, but **5c** afforded an intractable mixture. Similar results were also obtained directly from the corresponding azlactones without isolating **5**. It is noteworthy that the attempted acetylation of **6a** and **8a** with acetic anhydride and gl. acetic acid led to the formation of the parent azlactone **1c** ($R^1 = Ph$), thereby disproving the structure **7** ($R^2 = Ac$) proposed earlier[3]. The attempted cyclisation of the hydrazide **5a** to 2-imidazolin-5-one (**9**, $R^1 = Ph$; $R^2 = H$) by heating with gl. acetic acid gave a mixture in which only the presence of the azlactone **1c** ($R^1 = Ph$) was discernible by TLC and IR spectrum. Contrary to the earlier findings[2], the pyrazolidinone **8** ($R^1 = R^2 = Ph$) could not be isolated when a mixture of **1c** ($R^1 = Ph$) and phenylhydrazine was heated in ethanol for 4 hr. Instead, the phenylhydrazide **5b** and a small amount of 1-anilino-2-imidazolin-5-one (**9a**) were isolated. The latter was obtained in a better yield when the reaction was carried out in gl. acetic acid or a mixture of **1c** ($R^1 = Ph$) and **2** ($R^2 = Ph$) was heated at 160-70°. The compound **1e** ($R^1 = 2$-furyl) when heated with hydrazine underwent hydrazinolysis and subsequent cyclisation to **9b**, but the product decomposed when the reaction was carried out in gl. acetic acid. The hydrazide **5d** gave an intractable mixture under similar conditions.

The products reported were characterised by elemental analyses and spectral data. Their physical constants are given in Table 1.



a, $R^1 = EtO$
b, $R^1 = PhNH$
c, $R^1 = Ph$
d, $R^1 = 3\text{-MeO-4-OH-}C_6H_3$
e, $R^1 = 2$-Furyl

a, $R^1 = Ph$; $R^2 = H$
b, $R^1 = R^2 = Ph$
c, $R^1 = 3\text{-MeO-4 HO-}C_6H_3$; $R^2 = H$
d, $R^1 = 3\text{-MeO-4 HO-}C_6H_3$; $R^2 = Ph$
e, $R^1 = 2$-Furyl; $R^2 = H$
f, $R^1 = 2$-Furyl; $R^2 = Ph$

Table 1—Characterisation Data of 2-Acylamino-3-acrylic Acid Hydrazides (**5**), 5-Arylmethylene-3-phenyl-1,2,5,6-tetrahydro-1,2,4-triazin-6-ones (**6**), 5-Aryl-4-benzoylaminopyrazolidin-3-ones (**8**) and 1-Anilino-4-arylmethylene-2-phenyl-2-imidazolin-5-ones (**9**)

Compd*	Yield (%)	m.p. (°C)	Mol. formula	Found (Calc.)		
				C	H	N
5a	90	140-42	$C_{16}H_{15}N_3O_2 \cdot \frac{1}{2}H_2O$	66.2	5.2	14.5
				(66.5	5.6	14.8)
5b	60	196-98	$C_{22}H_{19}N_3O_2$	74.0	5.3	11.7
				(73.5	5.2	11.7)
5c	82	190-91	$C_{17}H_{17}N_3O_4 \cdot H_2O$	59.2	5.5	12.0
				(59.6	5.2	11.7)
5d	80	215-16	$C_{23}H_{21}N_3O_4$	68.5	5.2	10.4
				(68.9	5.7	10.7)
5e	72	186-87	$C_{14}H_{13}N_3O_3$	62.0	4.8	15.5
				(61.8	4.7	15.4)
5f	70	189-90	$C_{20}H_{17}N_3O_3$	69.2	4.9	12.1
				(69.4	5.0	12.5)
6a	53	175-76	$C_{16}H_{13}N_3O$	73.0	4.9	16.0
				(73.2	5.0	15.8)
6b	50	219-20	$C_{14}H_{11}N_3O_2$	66.4	4.4	16.6
				(66.4	4.4	16.6)
6c	74	188-90	$C_{17}H_{15}N_3O_3$	66.3	4.9	13.6
				(66.1	4.6	13.4)
8a	44	227-28	$C_{16}H_{15}N_3O_2$	68.3	5.3	15.0
				(68.1	5.4	15.2)
8b	40	224-25	$C_{14}H_{13}N_3O_3 \cdot H_2O$	58.5	4.5	14.3
				(58.1	5.1	14.5)
9a†	53	203-5	$C_{22}H_{17}N_3O$	77.9	5.0	12.4
				(77.9	5.0	12.0)
9b	50	240-41	$C_{20}H_{15}N_3O_2$	72.9	4.6	12.7
				(72.6	4.8	12.4)

*Due to inadequate solubility of the compounds their PMR spectra could not be recorded except for the compound **9a**.
†PMR($CDCl_3$/TMS): δ 6.52 (*s*, 1H, exchangeable with D_2O, PhN*H*), 6.94-8.03 (*m*, 16H, exocyclic methine proton and Ar − *H*).

Melting points are uncorrected. IR and PMR spectra were recorded on a Perkin-Elmer 720 and/or 257 spectrophotometer and JEOL FX-90Q instrument, respectively.

2-Acylamino-3-arylacrylic acid hydrazides (**5**): *General procedure*

An equimolar mixture of **1** (R^1 = aryl) and hydrazine in ethanol (20 ml/g of **1**), was heated under reflux for 15 min. When phenylhydrazine was used, the reaction was also carried out in benzene using a catalytic amount of gl. acetic acid and heating the mixture only for 10 min. Concentration of the reaction mixture gave a solid which on recrystallisation from aq. ethanol afforded **5** (Table 1); IR(nujol) of **5b**: 3300, 3200 (NH), 1680 (CO, amide), 1650 (C=C) cm^{-1}.

5-Arylmethylene-3-phenyl-1,2,5,6-tetrahydro-1,2,4-triazin-6-ones (**6**): *General procedure*

A mixture of N-unsubstituted 2-benzoylamino-3-arylacrylic acid hydrazide (**5**, R^2 = H) and sodium hydroxide in a molar ratio of 1:1.5 was suspended in distilled water (25 ml/g of **5**). The reaction mixture was boiled for 5 min, cooled and acidified with conc. HCl to give a solid which was filtered and recrystallised from aq. ethanol to give **6** (Table 1); IR (nujol) of **6a**: 3300, 3200 (NH), 1660 (CO, triazinone) cm^{-1}.

5-Aryl-4-benzoylaminopyrazolidin-3-ones (**8**): *General procedure*

A mixture of N-unsubstituted hydrazide (**5**, R^2 = H) and hydrazine hydrate in a molar ratio of 1:10 in ethanol (1 ml/g of **5**) was heated under reflux for 30 min, and diluted with water to give a solid which was filtered and recrystallised from aq. ethanol to give **8** (Table 1); IR(nujol) of **8a**: 3300, 3200 (NH), 1710 (CO, pyrazolidinone), 1650 (CO, amide) cm^{-1}.

1-Anilino-4-arylmethylene-2-phenyl-2-imidazolin-5-ones (**9**): *General procedure*

An equimolar mixture of **1** (R^1 = aryl) and

phenylhydrazine was heated at 160-70° for 20 min, extracted with hot benzene and the solution stripped of solvent to give an yellow solid which on recrystallisation from aq. ethanol furnished **9** (Table 1); IR(nujol) of **6a**: 3300, 3200 (NH), 1660 imidazolinone), 1640 (C=C) cm^{-1}.

The authors are thankful to the authorities of BHU for facilities, and to the Government of Iran for the award of a research fellowship to one of them (AMT).

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Convenient Preparation of Arylacetic Acids by Tetracarbonylcobaltate Anion Catalysed Carbonylation†

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Tetracarbonylcobaltate catalyst $[Co(CO)_4]^-$, has been prepared in 6 hr by pressure carbonylation of cobalt salt in aq alkaline solution containing cyanide catalyst. Carbonylation of arylchloromethyl derivatives in aq methanolic solutions using this catalyst affords arylacetic acids in good yields.

Carbonylation of benzyl chloride to give esters of phenylacetic acid was earlier carried out[1,2] in anhydrous solvents, and the catalyst, tetracarbonylcobaltate anion $[Co(CO)_4]^-$, which in turn was prepared from $Co_2(CO)_8$. More recently, phase transfer catalysed carbonylation reaction[3,4] in aq. alkaline solution has been reported to give good yield of phenylacetic acid. However, $Co_2(CO)_8$ used in these reactions is itself obtained by high pressure and high temperature carbonylation of cobalt salts.

In our attempts to prepare commercially important arylacetic acids via carbonylation reaction, we were interested in the preparation of $[Co(CO)_4]^-$ catalyst in aq alkaline solution. Cobalt salts are known to get carbonylated in aqueous solutions[5], but rather sluggishly (20-70 hr). We envisaged that since CO is a reducing agent in the reaction, the increase in CO pressure should result in faster carbonylation of cobalt salt. Based on this assumption, aq alkaline solutions of cobalt salts were reacted with CO under pressure (10 bar) at room temperature and it was observed that $[Co(CO)_4]^-$ could be prepared in 6-8 hr as compared to 20-70 hr in the earlier preparation.

In a typical procedure for carbonylation reaction, a solution of $Co(NO_3)_2.6H_2O$ (1.82 g) in water (12.5 ml) was treated with KOH (2.86 g) and KCN (0.8 g) and reacted with CO (10 bar) by rapidly stirring for 6 hr at 30°. The yellow coloured catalyst solution was taken in methanol (100 ml) and to it were added while stirring benzyl chloride (12.6 g) and aq NaOH (6*N*, 33 ml) dropwise during 4 hr at 55° under CO atmosphere. The reaction was continued for 2 hr and methanol distilled off. The cooled reaction mixture was filtered and the clear filtrate was acidified with conc HCl to give phenylacetic acid (11.5 g, 85%). Several arylchloromethyl derivatives were subjected to similar carbonylation reaction to give the corresponding arylacetic acids in good yields (Table 1).

Table 1—Preparation of Various Phenylacetic Acids by Carbonylation of Benzyl Chloride[a,b]

Sl No.	Benzyl chloride derivative (g)	Temp (°C)	Phenylacetic[c] acid derivative (g)	Yield (%)
1	Benzyl chloride (12.6)	55	Phenylacetic acid (11.5)	85
2	*p*-Chloro (12.5)	55	*p*-Chloro (11.2)	84
3	*m*-Benzyloxy (12.0)	55	*m*-Benzyloxy (9.35)	75
4	*m*-Methoxy (11.6)	55	*m*-Methoxy (9)	73
5	*p*-Methoxy (12.5)	10	*p*-Methoxy (6.63)	50
6	*p*-Methyl (11.5)	50	*p*-Methyl (9.0)	73
7	*p*-Ethyl (12.0)	50	*p*-Ethyl (10.2)	80
8	*p*-Isopropyl (10.0)	50	*p*-Isopropyl (7.0)	66
9	2,5-Dimethoxy (6.4)	50	2,5-Dimethoxy (5.4)	80
10	*p*-Isobutyl (9.10)	55	*p*-Isobutyl (6.82)	76
11	*m*-Phenoxy (10.9)	55	*m*-Phenoxy (8.0)	70

[a]1-Chloromethylnaphthalene on similar carbonylation afforded naphthalene-1-acetic acid in 66% yield.

[b]α-Methylbenzyl chloride yielded α-methylbenzyl methyl ether in 77% yield.

[c]All the products were fully characterised by analytical, PMR and mass data

It is clear that α-methylbenzyl chloride did not undergo carbonylation, instead reacted with methanol to give the corresponding methyl ether. Although, benzyl chloride reacted with CO to give phenylacetic acid in 85% yield, it was found that 5-10% of benzyl methyl ether was also formed. Therefore, methanolysis is a competing reaction to the carbonylation under these conditions. The increase in steric hindrance at the benzylic carbon atom is expected to decrease the rate of attack of sp^3-hybridised $[Co(CO)_4]^-$, which is the rate-determining step in the carbonylation reaction. Hence, α-methylbenzyl chloride reacted predominantly with methanol to give its methyl ether.

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Reactions of 1,3-Diamino- & 1,4-Diamino-benzenes with 1-Aryl-3-phenyl-prop-2-yn-1-ones

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1,3-Diamino-(IIa)- and 1,4-diamino-(IIb)-benzenes react with 1-aryl-3-phenyl-prop-2-yn-1-ones (Ia-c) in 1:1 molar proportion to give 1-aryl-3-phenyl-3-(3′-aminoanilino)-(IIIa-c)-, 1-aryl-3-phenyl-3-(4′-aminoanilino)-(IIId-f)-prop-2-en-1-ones together with 1,3-phenylene-(IVa-c)- and 1,4-phenylene-(Va-c)-di-[1-aryl-3-amino-3-phenyl-prop-2-en-1-ones], respectively. The structures of the products are based on analytical and spectral data.

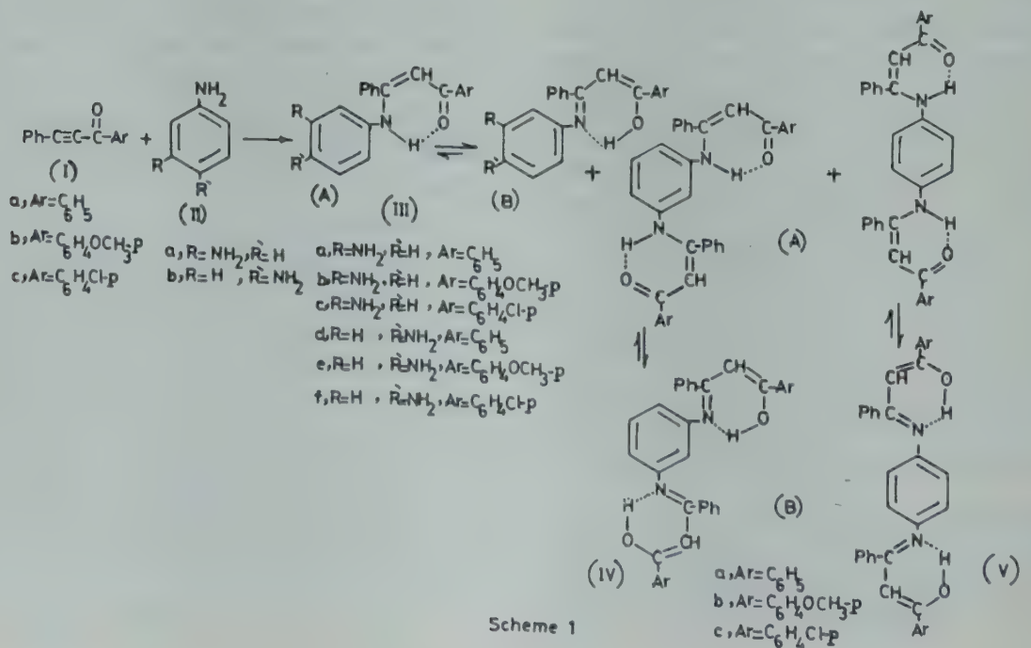
We recently reported[1] that 1,2-diaminobenzenes react with 1-aryl-3-phenyl-prop-2-yn-1-ones (I) to give the corresponding benzo-1,5-diazepine derivatives. This reaction has now been extended to 1,3-diamino-(IIa)- and 1,4-diamino-(IIb)-benzenes.

Addition of an equimolar amount of 1,3-diamino-(IIa)- or 1,4-diamino-(IIb)-benzene to each of the 1-aryl-3-phenyl-prop-2-yn-1-ones (Ia-c) led mainly to the monoadducts, 1-aryl-3-phenyl-3-(3′-aminoanilino)-(IIIa-c)- or 1-aryl-3-phenyl-3-(4′-aminoanilino)-(IIId-f)-prop-2-en-1-ones together with small amount of the di-adducts, namely, 1,3-phenylene-(IVa-c)- or 1,4-phenylene-(Va-c)-di-[1-aryl-3-amino-3-phenyl-prop-2-en-1-ones], respectively (Scheme 1; Table 1).

The structures of the monoadducts (IIIa-f) and the diadducts (IVa-c) and (Va-c) were confirmed by their analytical data, IR, UV, PMR and mass spectra. The existence of a free amino group in IIIa-f was confirmed by the appearance of two peaks in their IR in the NH region due to ν_s and ν_{as} NH. On the other hand, the diadducts (IVa-c) and (Va-c) exhibited in their IR spectra a broad band at 3460 cm^{-1} attributable to the partially bonded NH and/or OH group. The PMR spectra of IIIa-f displayed signals at δ 6.12-6.05(*s*,1H,olefinic), 3.6(*bs*,2H,NH_2 exchangeable with D_2O) and at 12.8-13.15 (*bs*,1H,OH), beside the signals due to aromatic protons. The PMR spectra of IVa-c and Va-c exhibited signals at δ 6.05(*s*,2H,olefinic),13.16-12.68(*bs*,2H,2 OH) in addition to the signals due to the aromatic protons. The existence of IIIa-f and IVa-c and Va-c in $CDCl_3$ as the enol-tautomer (B) is inferred from the appearance of a broad signal at δ 13.16-12.68, which is consistent with an enolic-OH proton rather than a proton attached to nitrogen as in the keto-form (A). Such a contribution was supported by the ^{13}C NMR which displayed signals characteristic of C = O and C = N groups.

When the reaction of I and II was carried out in 2:1 molar proportion, the diadducts (IV) and (V) were the major products and the monoadducts (III) were obtained as the minor products. This means that the conversion of monoadducts into the diadducts is not fairly complete. This may be attributed to the diminished nucleophilicity of the amino group as a result of electron attraction of the en-one system.

Melting points are uncorrected. 1-Aryl-3-phenyl-



Scheme 1

Table 1—Physical Data of 1-Aryl-3-phenyl-3-(3′-aminoanilino)-(IIIa-c)-, 1-Aryl-3-phenyl-3-(4′-aminoanilino)-(IIId-f)-prop-2-en-1-ones, 1,3-Phenylene-(IVa-c)- and 1,4-phenylene-(Va-c)-di-[1-aryl-3-amino-3-phenyl-prop-2-en-1-ones]

Compd	m.p.°C (solvent)	Yield %	Mol.formula (M^+)	Calc (found)%		
				C	H	N
IIIa	79-80 (P_c)	70	$C_{21}H_{18}N_2O$ (314.374)	80.2 (80.4	5.8 5.7	8.9 8.7)
IIIb	181-82 (E)	76	$C_{22}H_{20}N_2O_2$ (344.4)	76.7 (77.1	5.9 6.1	8.1 8.6)
IIIc	166-67 (B)	78	$C_{21}H_{17}ClN_2O$ (348.826)	72.3 (72.6	4.9 5.2	8.0 8.2)
IIId	154-55 (E)	68	$C_{21}H_{18}N_2O$ (314.374)	80.2 (80.4	5.8 6.0	8.9 9.0)
IIIe	189-90 (B)	71	$C_{22}H_{20}N_2O_2$ (344.4)	76.7 (77.3	5.9 6.0	8.1 7.8)
IIIf	206-7 (E)	69	$C_{21}H_{17}ClN_2O$ (348.826)	72.3 (72.4	4.9 5.1	8.0 7.8)
IVa	168-69 (P_c + B)	20	$C_{36}H_{26}N_2O_2$ (520.604)	83.0 (83.4	5.4 5.0	5.4 5.3)
IVb	187-88 (P_c + B)	22	$C_{38}H_{32}N_2O_4$ (580.656)	78.6 (78.7	5.6 5.8	4.8 4.6)
IVc	172-73 (*n*-butanol)	18	$C_{36}H_{26}Cl_2N_2O_2$ (589.508)	73.3 (73.3	4.4 4.7	4.8 4.3)
Va	251-52 (DMSO)	18	$C_{36}H_{26}N_2O_2$ (520.604)	83.0 (83.6	5.4 5.5	5.4 5.7)
Vb	256-57 (C)	19	$C_{38}H_{32}N_2O_4$ (580.656)	78.6 (78.7	5.6 5.8	4.8 4.6)
Vc	283-84 (C)	23	$C_{36}H_{26}Cl_2N_2O_2$ (589.508)	73.3 (73.4	4.4 4.6	4.8 4.3)

E = Ethanol; B = benzene; P_c = petrol(80-100°); and C = chloroform.

prop-2-yn-1-ones (Ia-c) were prepared by the oxidation of corresponding 1-aryl-3-phenyl-prop-2-yn-1-ols according to the method outlined by Barker *et al.*[2].

General procedure for addition of 1,3-*diamino*-(IIa)-*and* 1,4-*diamino*-(IIb)-*benzenes to* 1-*aryl*-3-*phenyl*-2-*yn*-1-*ones*(Ia-c)

To a solution of I (0.005 mol) in ethanol (20 ml) was added a solution of 1,3-diamino-(IIa)- or 1,4-diamino-(IIb)-benzene (0.005 mol) in ethanol (10 ml). The reaction mixture was refluxed for 24 hr, concentrated under reduced pressure, cooled to room temperature and the precipitated solid fractionally crystallised from suitable solvent(cf. Table 1) to give IIIa-c or IIId-f. The solid remained after separation of IIIa-c or II-Id-f was crystallised from appropriate solvent(cf. Table 1) to give IVa-c or Va-c.

Repeating these reactions using (0.01 mol) of I and (0.005 mol) of IIa or IIb gave mainly the diadducts (IVa-c) or (Va-c) together with a minor amount of the monoadducts (IIIa-c) or (IIId-f) and some of unchanged (Ia-c) and (IIa) or (IIb). IIIa:IR(KBr):1610 (C = O and/orC = N),3220,3360,3460(OH and/or NH); UV(ethanol):378(logε 4.29),257(logε 4.12), 224(logε 4.30); PMR($CDCl_3$):δ12.95(*bs*,1H,OH), 8.2-6.13(*m*,14H,ArH), 6.12(*s*,1H,CH =),3.6(*bs*,-2H,NH_2); *m/z*(%): 314(M^+, 31),209(M^+-PhCO,-100),105(PhCO,19).

IIId: IR(KBr):1595(C = O and/or C = N),3220,-3330,3450(OH and/or NH); UV(ethanol):388(logε 4.30),310(logε 4.00),252(logε 4.30);PMR($CDCl_3$): δ13.15(*bs*,1H,OH),8.2-6.2(*m*,14H,ArH),6.1(*s*,1H,-CH =),3.55(*bs*,2H,NH_2); ^{13}C NMR($CDCl_3$): 188.7-(C = O),162.1(C = N),95.5(CH =); *m/z*(%):314(M^+,-94),209(M^+-PhCO,83),77(Ph,100).

IVa: IR(KBr):1595(C = O and/or C = N),3440(OH and/or NH); UV(ethanol): 384(logε 4.92),256(logε 4.69),230(logε 4.61); PMR($CDCl_3$):δ12.82(*br*,2H,-2OH),8.18-6.3(*m*,24H,ArH),6.15(*s*,2H,2 CH =); ^{13}CNMR($CDCl_3$):189.3(C = O),160.8(C = N),97.0-(CH =); *m/z*(%):520(M^+,16),415(M^+-PhCO,62),105 (PhCO,100).

Vb: IR(KBr):1590(C = O and/or C = N),3450(OH and/or NH); UV(ethanol): 406(logε 4.65),288(logε 4.34),260(logε 4.25); PMR($CDCl_3$):δ12.71(br,2H,-2-OH),7.98-6.52(*m*,22H,ArH), 3.85(*s*,6H,2-OMe); *m/z*(%):580(M^+,70),135(*p*-Me-OC_6H_4CO,100).

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Synthesis of 2-Aryloxy-3-cyclohexyl-6-(1,1-dimethylethyl)-3,4-dihydro-2*H*-1,3,2-benzoxazaphosphorine-2-oxides

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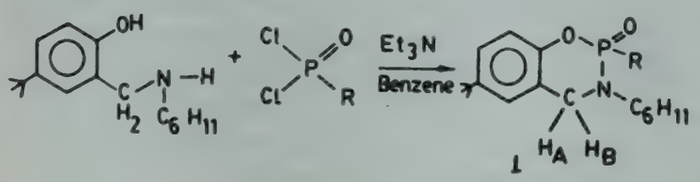
Several 2-aryloxy-3-cyclohexyl-6-(1,1-dimethylethyl)-3,4-dihydro-2*H*-1,3,2-benzoxazaphosphorine-2-oxides (**1a-m**) have been synthesized by the condensation of phenylphosphorodichloridates and phenylphosphoric dichloride with 2-cyclohexylamino-methyl-4-*t*-butylphenol.

In search for new anticancer drugs, synthesis of a large number of phosphorus heterocyclic compounds is reported in literature and some of them are found to possess significant antitumour activity[1-4]. To add to the extensive literature on phosphorus heterocycles, we report in this note the synthesis of cyclophosphamide[5] related derivatives, 2-aryloxy-6-*t*-butyl-3-cyclohexyl-3,4-dihydro-2*H*-1,3,2-benzoxazaphosphorine-2-oxides (**1a-m**).



1

R	R
1a C_6H_5O	**1h** 2,5-$(CH_3)_2$-C_6H_3O
1b 2-CH_3-C_6H_4O	**1i** 2-Cl-C_6H_4O
1c 3-CH_3-C_6H_4O	**1j** 4-Cl-C_6H_4O
1d 4-CH_3-C_6H_4O	**1k** 4-Cl-3-CH_3-C_6H_3O
1e 3,4-$(CH_3)_2$-C_6H_3O	**1l** 4-Cl-2-CH_3-C_6H_3O
1f 3,5-$(CH_3)_2$-C_6H_3O	**1m** C_6H_5
1g 2,3-$(CH_3)_2$-C_6H_3O	

Compounds (**1a-m**) were synthesized by the following general procedure adopted for the synthesis of **1a**: Phenylphosphorodichloridate (2.12 g, 0.01 mol) in dry benzene (20 ml) was added dropwise to a stirred solution of 2-cyclohexylaminomethyl-4-*t*-butylphenol (2.61 g, 0.01 mol) and triethylamine (2.05 g, 0.02 mol) in dry benzene (40 ml) at 15-20°C. The reaction mixture was stirred at room temperature for 2 hr and for another 2 hr at 40-60°C. Solid triethylamine hydrochloride which separated out was filtered off and the filtrate concentrated in a rota evaporator. The residue was crystallised from propan-2-ol-tetrahydrofuran (4:1) to afford **1a** as colourless crystals; yield 3 g (75%), m.p. 125-27°; IR (KBr): 1275 (P=O), 1227, 918 (P−O−C_{aryl}) and 1078, 688 (P−N−$C_{aliphatic}$); PMR (CCl_4): δ 1.26 (*s*, 9H), 1.39-1.90 (*m*, 11H), 4.32 (*d*, *J*= 7.0 Hz, H_A), 4.24 (*d*, *J*= 19.0 Hz, H_B) and 6.78-7.38 (*m*, 8H) (Table 1). The influence of a substituent in the benzene ring of the dichloridate did not show any quantitative effect on the rate of the cyclization and yields of the cyclized products.

In IR, compounds (**1**) exhibited[6-12] νP=O in the region 1296-1255, νP−O−C (aryl) in the region 1238-1203, 936-915 and νP−N−C (aliphatic) in the region 1088-1050, 720-686 cm^{-1}. The PMR spectra of **1** exhibited *t*-butyl protons at δ 1.24-

Table 1 – Physical Data of Benzoxazaphosphorine -2-oxides (**1a-m**)

Compd	Yield (%)	Reaction time (hr)	m.p.[c] (°C)	Mol. formula	Found (Calc.) (%) C	H
1a	75[a]	4	125-27	$C_{23}H_{30}O_3NP$	69.3 (69.2)	7.5 (7.5)
1b	73[b]	4½	97-99	$C_{24}H_{32}O_3NP$	70.1 (69.7)	7.5 (7.7)
1c	73[b]	4	130-32	$C_{24}H_{32}O_3NP$	69.5 (69.7)	7.9 (7.7)
1d	73[b]	4½	126-28	$C_{24}H_{32}O_3NP$	70.1 (69.7)	7.5 (7.7)
1e	73[b]	5	108-10	$C_{25}H_{34}O_3NP$	70.4 (70.3)	7.9 (8.0)
1f	70[b]	5	92-94	$C_{25}H_{34}O_3NP$	70.5 (70.3)	8.0 (8.0)
1g	70[b]	5	86-88	$C_{25}H_{34}O_3NP$	70.5 (70.3)	7.8 (8.0)
1h	75[b]	5	117-19	$C_{25}H_{34}O_3NP$	70.1 (70.3)	7.7 (8.0)
1i	67[b]	5½	98-100	$C_{23}H_{29}O_3NPCl$	63.9 (63.7)	6.6 (6.7)
1j	74[b]	4½	94-96	$C_{23}H_{29}O_3NPCl$	63.8 (63.7)	6.6 (6.7)
1k	71[b]	5	125-27	$C_{24}H_{31}O_3NPCl$	64.7 (64.4)	7.0 (6.9)
1l	67[b]	5	68-70	$C_{24}H_{31}O_3NPCl$	64.7 (64.4)	7.1 (6.9)
1m	78[a]	4½	112-14	$C_{23}H_{30}O_2NP$	72.2 (72.1)	7.7 (7.8)

Compounds recrystallized from (a) propan-2-ol + THF and (b) propan-2-ol

(c) Melting points reported are uncorrect.

1.32, aromatic protons around 6.56-7.70 and remaining cyclohexyl and methyl protons at usual chemical shifts. The methylene protons (H_A and H_B) exhibited a three-line signal due to coupling[13] with phosphorus in the region δ 3.82-4.40 (two doublets, 7-11 Hz, H_A; 13-20 Hz, H_B).

2-Cyclohexylaminomethyl-4-*t*-butylphenol and phenyl-phosphorodichloridates were prepared as described in literature[14,15] and phenylphosphonic dichloride was a commercial product (Fluka).

The authors are thankful to Dr Kurt L Loening, Nomenclature Director, Chemical Abstracts Service, for the nomenclature of these compounds and to Dr G V Subba Raju for helpful discussion.

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Synthesis & Antimicrobial Activity of 2-Arylideneamino-4-phenyloxazoles, Azetidinones, Thiazolidinones, Thiazolines & Some Spiroheterocycles

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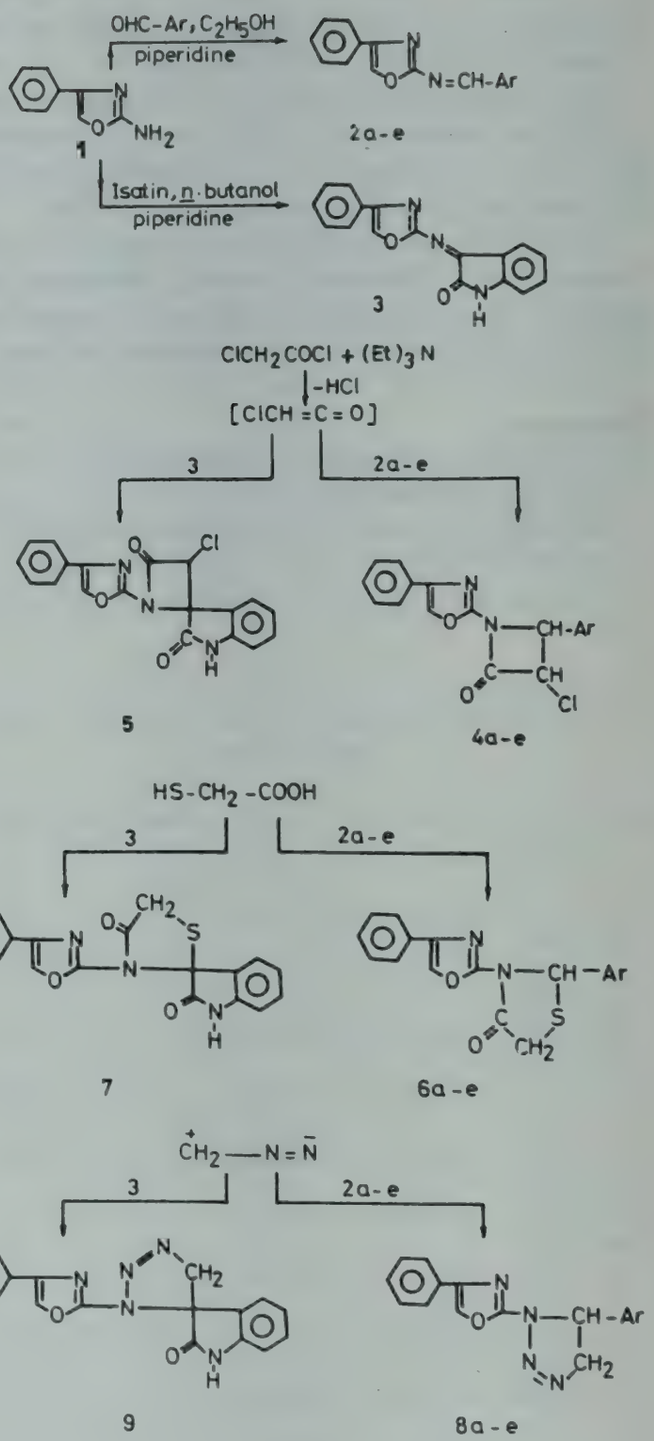
Condensation of 2-amino-4-phenyloxazole (**1**) with aromatic aldehydes gives 2-arylideneamino-4-phenyloxazoles (**2a-e**), while with isatin it gives 2-(isatin-3-ylideneamino-4-phenyloxazole (**3**). Cyclocondensation of **2** with monochloroacetyl chloride, mercaptoacetic acid and diazomethane affords azetidinones (**4a-e**), thiazolidinones (**6a-e**) and triazolines (**8a-e**), respectively, while that of **3** with these reagents gives spiroazetidinone (**5**), spirothiazolidenone (**7**) and spirotriazoline (**9**) respectively containing oxazole ring. The antimicrobial activity of some of these compounds against various strains of bacteria has been determined.

Because of our interest in the chemistry of oxazoles, azetidinones, thiazolidinones and triazolines, we have published a series of papers in these fields[1-6] and established the dipolar cyclocondensation of monochloroacetyl chloride, mercaptoacetic acid and diazomethane on the anils of biologically active compounds such as benzanilides[7], diarylsulphides and diarylsulphones[8-15]. The results obtained from these studies prompted us to investigate the dipolar cyclocondensation of the above reagents on the anils (**2**) of 2-amino-4-phenyloxazole (**1**). The results are reported in this note.

The compound **1** underwent facile condensation with aromatic aldehydes in abs. ethanol in the presence of piperidene as a basic catalyst giving 2-arylideneamino-4-phenyloxazoles (**2a-e**) in quantitative yield, while condensation of isatin with **1** in dry *n*-butanol in the presence of dry piperidene yielded the corresponding azomethine (**3**).

The anils **2a-e** and **3** on condensation with monochloroacetyl chloride in dry dioxane in the presence of triethylamine produced 4-aryl-3-chloro-1-(4-phenyl-2-oxazolyl)azetidin-2-ones (**4a-e**) and 3′-chloro-1′-(4″-phenyl-2″-oxazolyl)spiro[indoline-3,4′-azetidine]-2,2′-dione (**5**), respectively. Apparently this cyclocondensation proceeds via a ketene intermediate as reported in literature[16]. The cyclocondensation of mercaptoacetic acid with **2a-e** and **3** was carried out in dry benzene using Dean-Stark trap to give 2-aryl-3-(4-phenyl-2-oxazolyl)thiazolidin-4-ones (**6a-e**) and 3′-(4″-phenyl-2″-oxazolyl)-spiro[indoline-3,2′-thiazolidine]-2,4′-dione (**7**), respectively.Similarly, 1,3 dipolar cyclocondensation of **2a-e** and **3** with diazomethane proceeded in a dry dioxan-ether mixture at 15° for 15 days to produce 5-aryl-1-(4′-phenyl-2′-oxazolyl)-Δ^2-triazolines (**8a-e**) and 1-(4″-phenyl-2″-oxazolyl)spiro[indoline-3,5′-Δ^2-



triazolin]2-one (**9**), respectively. The characterization data of these products are given in Table 1.

The comparatively higher yield of **8b** can be attributed to the presence of nitro group in the aldehyde moiety of the corresponding aldimine (**2b**). The nitro group in **2** being in conjugation with the azomethine linkage exerts effect on the latter thereby facilitating the 1,3-dipolar cycloaddition of diazomethane on this center (azomethine). The structures of the compounds (**2-9**) were based on elemental analyses and characteristic IR data.

The antimicrobial of the prepared compounds was determined against a variety of microbes by

Table 1—Physical Data of the Compounds **2-9**

Compd.	Ar	Yield (%)	m.p. °C	Mol. formula	Found (%) (Calc)*		
					N	Cl	S
2a	*p*-OCH_3-C_6H_4	70	188	$C_{17}H_{14}N_2O_2$	10.1 (9.9	— —	— —)
2b	*p*-NO_2-C_6H_4	74	194	$C_{16}H_{11}N_3O_3$	14.1 (14.4	— —	—) —)
2c	*p*-Cl-C_6H_4	72	198	$C_{16}H_{11}N_2OC1$	9.9 (10.1	12.6 12.5	— —)
2d	*o*-OH-C_6H_4	68	152-53	$C_{16}H_{12}N_2O_2$	10.6 (10.4	— —	— —)
2e	*p*-$N(CH_3)_2$-C_6H_4	69	195	$C_{18}H_{17}N_3O$	14.4 (14.8	— —	— —)
3	———	53	166-67	$C_{17}H_{11}N_3O_2$	14.5 (14.2	— —	— —)
4a	*p*-OCH_3-C_6H_4	53	195	$C_{19}H_{15}N_2O_3Cl$	7.9 (8.15	10.0 9.7	— —)
4b	*p*-NO_2-C_6H_4	62	136-38	$C_{18}H_{12}N_3O_4Cl$	11.4 (11.6	9.6 9.4	— —)
4c	*p*-Cl-C_6H_4	65	180	$C_{18}H_{12}N_2O_2Cl_2$	7.8 (7.3	19.6 19.8	— —)
4d	*o*-OH-C_6H_4	57	128-30	$C_{18}H_{12}N_2O_3Cl$	8.3 (8.7	10.5 10.7	— —)
4e	*p*-$N(CH_3)_2$-C_6H_4	64	112-15	$C_{20}H_{18}N_3O_2Cl$	11.4 (11.1	9.7 9.4	— —)
5	———	63	138-40	$C_{19}H_{12}N_3O_2Cl$	11.5 (11.7	9.7 9.3	— —)
6a	*p*-OCH_3-C_6H_4	45	127-29	$C_{19}H_{16}N_2O_3S$	8.0 (8.2	— —	9.1 8.9)
6b	*p*-NO_2-C_6H_4	47	148-50	$C_{18}H_{13}N_3O_4S$	11.4 (11.7	— —	8.7 8.3)
6c	*p*-Cl-C_6H_4	42	122	$C_{18}H_{13}N_2O_2SCl$	7.9 (7.6	10.0 9.7	9.0 9.2)
6d	*o*-OH-C_6H_4	52	128-30	$C_{18}H_{14}N_2O_3S$	8.3 (8.6	— —	9.5 9.3)
6e	*p*-$N(CH_3)_2$-C_6H_4	55	132-35	$C_{20}H_{19}N_3O_2S$	11.5 (11.8	— —	8.8 8.3)
7	———	57	122-25	$C_{19}H_{13}N_3O_3S$	11.6 (11.9	— —	8.8 8.4)
8a	*p*-OCH_3-C_6H_4	69	125	$C_{18}H_{16}N_4O_2$	17.5 (11.9	— —	— —)
8b	*p*-NO_2-C_6H_4	78	124-26	$C_{17}H_{13}N_5O_3$	20.9 (20.7	— —	— —)
8c	*p*-Cl-C_6H_4	81	110-15	$C_{17}H_{13}N_4OCl$	17.3 (17.5	11.0 11.1	— —)
8d	*o*-OH-C_6H_4	75	120-22	$C_{17}H_{14}N_4O_2$	18.3 (18.2	— —	— —)
8e	*p*-$N(CH_3)_2$-C_6H_4	76	118-20	$C_{19}H_{19}N_5O$	21.0 (21.3	— —	— —)
9	———	73	142-45	$C_{18}H_{13}N_5O_2$	21.1 (21.6	— —	— —)

*All compounds gave satisfactory C and H analyses.

the literature method[17]. The compounds exhibited 10 to 50% inhibation at $10^{-3}M$ concentration. Compounds derived from **3** were more active than those derived from **2**. Compounds **4d, 6d, 9a, 8d, 5** and **7** were found to be more active against *Staphylococcus aureus, Escherichia coli, Micrococcus luteus, Klebssiela pneumoniae* and *Bacilleus areus*, and **4b, 4d, 6d, 6c, 8a, 8c, 8e, 5, 7** and **9** against all the above microbes and *Serratie* sp.

The reaction period and the purity of the compounds were determined by TLC. Melting points are uncorrected. IR spectra in KBr were recorded on a Unicam SP 200 G spectrophotometer (ν_{max} in cm^{-1}).

2-Amino-4-phenyloxazole (**1**) was prepared according to the method described in the literature[19].

2-Arylideneamino-4-phenyloxazoles (*2a-e*)

A mixture of **1** (0.01 mol) and the appropriate aromatic aldehyde (0.015 mol) in abs. ethanol or *n*-butanol (50 ml) was heated under reflux for 2 hr in the presence of 2 drops of dry piperidine, and the reaction mixture worked-up as usual to get **2** (Table 1).

2-(3-Isatylidene)amino-4-phenyloxazole (**3**)

An equimolar mixture of isatin and **1** was dissolved in abs. ethanol and the rection mixture refluxed for 5 hr, and concentrated to get **3** which was filtered, washed with ethanol and recrystallized from ethanol.

4-Aryl-3-chloro-1-(4-phenyl-2-oxazolyl)azetidin-2-one (**4a-e**)

To a well stirred solution of **2a-e** (0.01 mol) and triethylamine (0.02 mol) in dry dioxane (100 ml) was added monochloroacetyl chloride (0.02 mol) dropwise at room temperature, the reaction mixture stirred for 5 hr and worked-up as usual to get **4** (Table 1).

3'-Chloro-1'-(4''-phenyl-2''-oxazolyl)spiro[indoline-3,4'-azetidine]-2,2'-dione (**5**)

It was prepared by a similar procedure employed for the preparation of **4** using **3** instead of **2**.

2-Aryl-3-(4'-phenyl-2-oxazolyl)thiazolidine-4-ones (**6a-e**; Table 1)

To a solution of **2a-e** (0.01 mol) in dry benzene was added mercaptoacetic acid (0.01 mol) and the reaction mixture refluxed using a water separator. At the end of the reaction, benzene was removed under reduced pressure, the residue dissolved in ether and seeded to get **6**.

3'-(4''-Phenyl-2''-oxazolyl)spiro[indoline-3,2'-thiazolidine]-2,4'-dione (**7**)

It was prepared following the above procedure using **3**.

5-Aryl-1-(4'-phenyl-2'-oxazole)-Δ^2-triazolines (**8a-e**; Table 1)

A solution of **2a-e** (lg) in dry dioxane was treated with an excess of dry and cold etherial diazomethane. The reaction mixture was kept at $-15°$ during which fresh amounts of etherial diazomethane were added. The reaction was monitored for 15 days by TLC. Thereafter, ether was evaporated and the product filtered off, washed with light pet. ether (40-60°) and crystallized from a proper solvent.

5'-(4''-phenyl-2''-oxazolyl)spiro[indoline-3,1'-Δ^2-triazolin]-2-ones (**9**)

It was prepared by the same procedure as described above using **3**.

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5-Substituted 2-{4-[4-(*m*-Chlorophenyl)-piperazin-1-yl]-7-trifluoromethyl-quinolin -3-yl}-1,3,4-oxadiazoles as Hypotensive Agents

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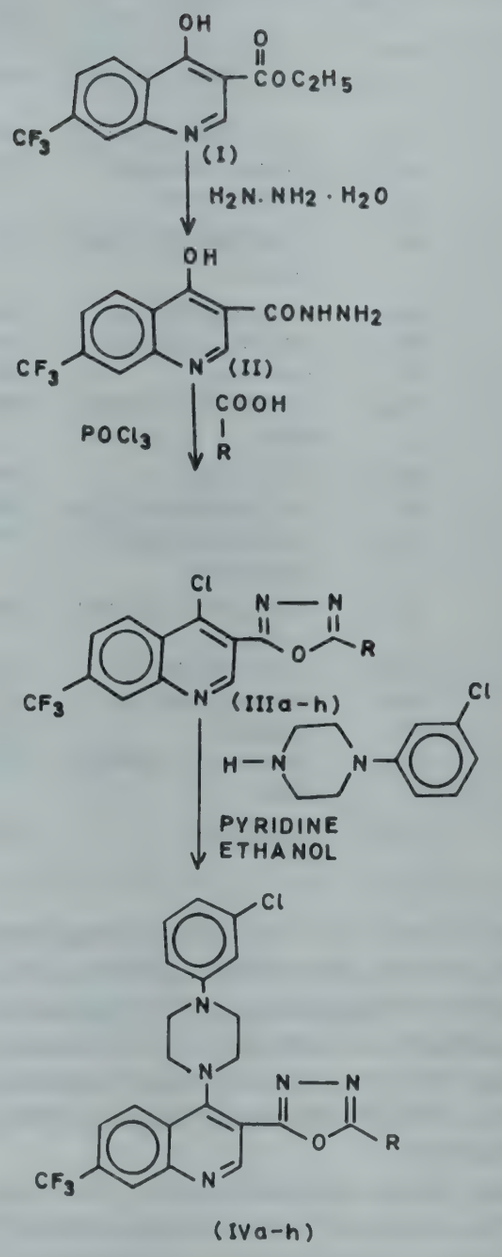
The title compounds (IVa-h) have been synthesized starting from ethyl 7-trifloromethyl-4-hydroxyquinoline-3-carboxylate and evaluated for their hypotensive activity.

Substitution pattern in quinoline nucleus plays a pivotal role in delineating the biological activity; substitution at position-8 imparts amoebicidal, antimalarial and antifungal activities[1–3]. Cardiovascular activity has also been reported for substituted quinolines[4–5]. Furthermore, piperazines and oxadiazole possess hypotensive activity[6]. Thus, it was thought worthwhile to synthesize quinoline derivatives (IV) bearing piperazine and oxadiazole moieties and evaluate their hypotensive activity. The results are reported in this note. The reaction sequence leading to the formation of IV is shown in scheme 1.

The required ethyl 7-trifluoromethyl-4-hydroxyquinoline-3-carboxylate (I) was prepared by the reported method[7]. Treatment of I with hydrazine hydrate under reflux gave 7-trifluoromethyl-4-hydroxyquinoline-3-carboxylic acid hydrazide (II). Its structure was derived from elemental analysis and PMR spectral data. Among the significant features of its PMR spectrum were the appearance of the signals δ 1.45 (singlet) and at 0.70 (singlet). The reaction of II with aromatic acids and benzoylamino acids gave the oxadiazoles(III). It is worthmentioning that $POCl_3$ replaces the hydroxy group at position-4 with Cl with the simultaneous formation of an oxadiazole ring. The absence of signals due to NH and NH_2 protons in the PMR spectrum of the product confirms that the hydrazino group of II undergoes cyclization. Furthermore, the signal due to OH proton (present at δ 10.10 as a sharp singlet in compound II) was also absent.

The oxadiazoles (III) on condensation with 1-(*m*-chlorophenyl)piperazine afforded the title compounds (IV) (Table 1).



SCHEME 1

Hypotensive activity

The effects of the compounds IIIa-h and IVa-h were studied on resting blood pressure and pressor responses evoked either by carotid occlusion (CO) for 15 or 30 sec or by intravenous injection of noradrenaline (NA) (1-2 μg/kg) in chloralose (80 mg/kg i.v.) anesthetized cats. The tests were carried out at a dose of 1 mg/kg intravenously.

Among the compounds screened, IVd, IVf and IVg exhibited transient hypotension [fall in blood pressure by 10 (dalayed), 50 (immediate) and 60 (immediate)mm Hg respectively] which lasted for

Table 1—Characterisation Data of 2-(4-Chloro-7-trifluoromethyl-5-substituted quinolin-3-yl)-1,3 4-oxadiazoles (IIIa-h) and 2-{4-[4-(*m*-Chlorophenyl)piperazin-1-yl]-7-trifluoromethylquinolin-3-yl}-1,3,4-oxadiazoles (IVa-h)

Compd	R	m.p. °C	yield (%)	Mol. formula	N (%)	
					Found	Calc.
IIIa	$-C_6H_4NHCOC_6H_5(p)$	230	65	$C_{25}H_{14}N_4O_2F_3Cl$	11.7	11.3
IIIb	1-Benzoylamino-2-(3-indolyl)ethyl	285	60	$C_{29}H_{19}N_5O_2F_3Cl$	12.3	12.5
IIIc	$-CH_2NHCOCH_2NHCOC_6H_5$	176	55	$C_{22}H_{15}N_5O_3F_3Cl$	14.7	14.3
IIId	$-CH_2NHCOC_6H_5$	328	55	$C_{20}H_{12}O_2N_4F_3Cl$	12-6	12.9
IIIe	5-Methyl-2-indolyl	156	50	$C_{21}H_{12}N_4F_3OCl$	13.4	13.1
IIIf	2-Indolyl	287	55	$C_{20}H_{10}N_4OF_3Cl$	13.7	13.5
IIIg	1-Amino-2-(3-indolyl)-ethyl	296	60	$C_{23}H_{15}N_5OF_3Cl$	14.5	14.9
IIIh	$C_6H_4(OH)(o)$	159	50	$C_{18}H_9N_3O_2F_3Cl$	10.4	10.7
IVa	$-C_6H_4NHCOC_6H_5(p)$	338	50	$C_{35}H_{26}N_6O_2F_3Cl$	13.4	12.8
IVb	1-Benzoylamino-2-(3-indolyl)ethyl	356	45	$C_{39}H_{31}N_7O_2F_3Cl$	13.8	13.6
IVc	$-CH_2NHCOCH_2NHCOC_6H_5$	287	40	$C_{32}H_{27}N_7O_3F_3Cl$	15.2	15.1
IVd	$-CH_2NHCOC_6H_5$	256	55	$C_{30}H_{24}N_6O_2F_3Cl$	14.4	14.2
IVe	5-Methyl-2-indolyl	222	70	$C_{31}H_{24}N_6OF_3Cl$	14.4	14.3
IVf	2-Indolyl	247	55	$C_{30}H_{22}N_6OF_3Cl$	14.8	14.6
IVg	1-Amino-2-(3-indolyl)-ethyl	283	50	$C_{33}H_{27}N_7OF_3Cl$	15.4	15.6
IVh	$C_6H_4OH(o)$	247	40	$C_{28}H_{21}N_5O_2F_3Cl$	12.5	12.7

less than 10 min. On the contrary, compounds IVb and IVe induced hypertension [increase in blood pressure by 30 (immediate) and 90 (immediate) mm Hg respectively] of short duration. Furthermore, no significant effect on evoked pressor responses (CO and NA) could be observed with any of the compounds tested.

Melting points are uncorrected. TLC was performed on silica gel G plates using benzene-methanol as solvent. IR spectra were recorded in KBr on a Perkin-Elmer 157 spectophotometer (ν_{max} in cm^{-1}) and 60 MHz PMR spectra in TFA on an EM-360 spectrometer using TMS as internal standard (chemical shifts in δ, ppm).

7-Trifluoromethyl-4-hydroxyquinoline-3-carboxylic acid hydrazide (II)

A mixture of I and hydrazine hydrate (100 ml) was refluxed for 16 hr, concentrated and poured into ice cold water. The solid that separated out was filtered and recrystallized from ethanol, m.p. 255°, yield 70%; IR: 3400 (NH), 3500 (OH) and 1720 cm^{-1} (CO); PMR: 7.80-8.50 (*m*, 4H, Ar-*H*), 10.10 (*s*, 1H, Ar-O*H*), 1.45 (*s*, 1H, CON*H*), 0.70 (*s*, 2H, CONHNH_2).

2-(4-Chloro-7-trifluoromethyl-2-substituted-quinolin-3-yl)-1,3,4-oxadiazoles (IIIa-h)

A mixture of II (0.01 mol) and N-benzoylglycine (0.01 mol) in $POCl_3$ was refluxed for 10 hr, cooled, poured into ice water and neutralized with $NaHCO_3$ solution (20%). The resultant solid was filtered, washed with water and recrystallized from ethanol to give IIId, m.p. 328°, yield 55%; IR: 1620 (C=N); PMR: 7.30-9.0 (*m*, 9H, Ar-*H*), 9.20 (*s*, 1H, NHCO), 1.50 (*s*, 2H, CH_2NH).

5-Substituted 2-{4-[4-(m-chlorophenyl)piperazin-1-yl]-7-trifluoromethylquinolin-3-yl}-1,3,4-oxadiazoles (IVa-h)

To a solution of IIId (0.01 mol) in abs. ethanol (50 ml) were added 1-(*m*-chlorophenyl)piperazine (0.01 mol) and a few drops of pyridine. The reaction mixture was refluxed for 8 hr and poured into water and the resultant solid filtered and recrystallized from ethanol-water to give IVd, m.p. 256°, yield 55%; PMR: 7.20-8.90 (*m*, 13H, Ar-*H*), 9.20 (*s*, 1H, NHCO), 1.75 (*s*, 2H, CH_2-NH), 2.35 (*m*, 8H, methylene protons of piperazine).

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Studies in the Friedel-Crafts Reaction: Part III—Friedel-Crafts Reaction of 2,3-Diarylpentanedioic Anhydride

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2,3-Diarylpentanedioic anhydrides (**4**) on Friedel-Crafts reaction in nitrobenzene in the presence of $AlCl_3$ give α-aryl-1-oxo-indane-3-acetic acids (**7**). In no case the corresponding tetralones are obtained. The ring size has been determined by PMR and ^{13}CNMR spectral studies. The anhydrides (**4**) react with anisole in the presence of $AlCl_3$ in nitrobenzene to form 5-anisyl-2,3-diaryl-5-oxo-pentanoic acids (**8**). The position of anisyl residue has been determined by $NaBH_4$ reduction of the methyl ester of **8a** and examining the PMR spectrum of the reduced ester. A probable mechanism for these reactions has been discussed.

2,3-Diphenylpentanedioic anhydride (**4a**) on treatment with $AlCl_3$ in nitrobenzene is reported to give **7a**[1]. However, choice between the isomeric indanones **6** and **7** was not clearly made. Similarly, the intermolecular Friedel-Crafts reaction of **4** is also known[2]. During our investigations on the Friedel-Crafts reaction of aryl substituted cyclic anhydrides, we found the anhydride **4** to be an interesting substrate for the reaction as its intramolecular Friedel-Crafts reaction would lead to isomeric indanones **6** or **7** or tetralone **5**. Similarly, its intermolecular Friedel-Crafts reaction could lead to positionally isomeric δ-keto acids **8** or **9**. Hence, we undertook a systematic study of the Friedel-Crafts reaction of **4**. The results are reported in this note.

When anhydride **4a** was treated with $AlCl_3$ in nitrobenzene, **7a** was obtained. The structure of **7a** was assigned on the basis of its PMR and ^{13}CNMR spectra. To prove the presence of an indanone ring in **7a**, model indanone-3-acetic acid (**12**)[3] and 1-tetralone-4-carboxylic acid (**13**)[4] were prepared by the known routes and their ^{13}CNMR data compared with those of **7a** (Table 1). The carbonyl carbon in **12** and **7a** appeared at δ 204.63 and 204.6, respectively, while the same in **13** appeared at 197.59. The carboxyl carbon of **12**, **7a** and **13** appeared at δ 173.13, 173.68 and 177.79, respectively. These values clearly indicated the indanone structure for **7a**, and also that the size of the ring exerts a profound effect on the ^{13}C shift of a carbonyl carbon.

Similarly, the anhyhdride **4c** on treatment with $AlCl_3$ gave **7c**. Compound **4b** bearing a methoxyl group on the 2-phenyl ring was prepared to see the possibility of tetralone formation, as in this case cyclisation of 5-carboxyl group with 2-aryl ring is electronically more favourable. However, **4b** gave indanone **7b**. Thus, it appeared that an indanone formation is kinetically more favourable than the corresponding tetralone formation.

The probable explanation for this behaviour is sought in the structure of the $AlCl_3$-complex of **4**. The complex would take the preferred conformation **11** in which the acyl carbonium ion is in a sterically suitable position with respect to the 3-phenyl

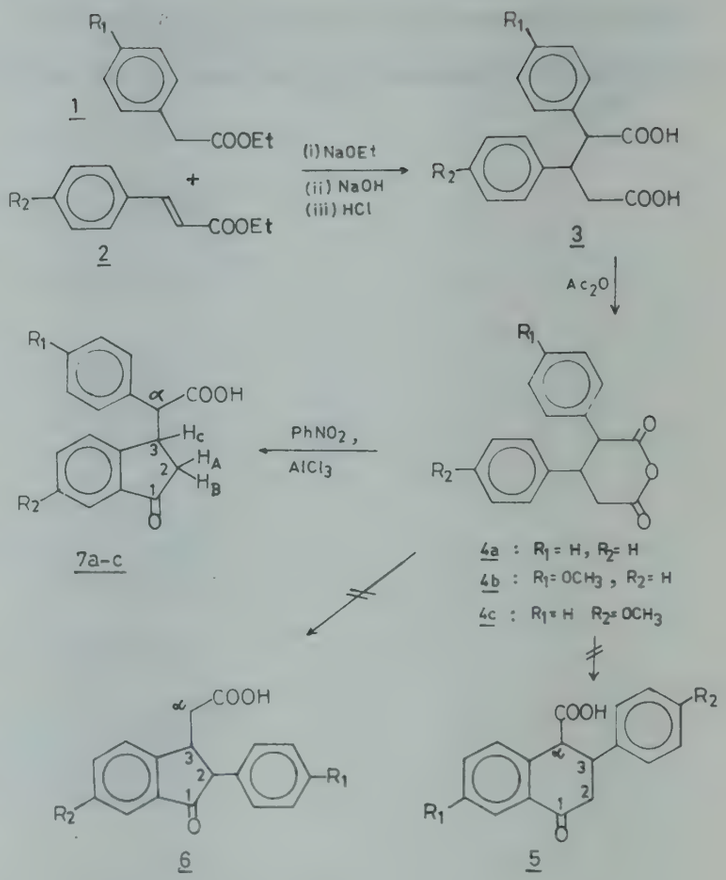
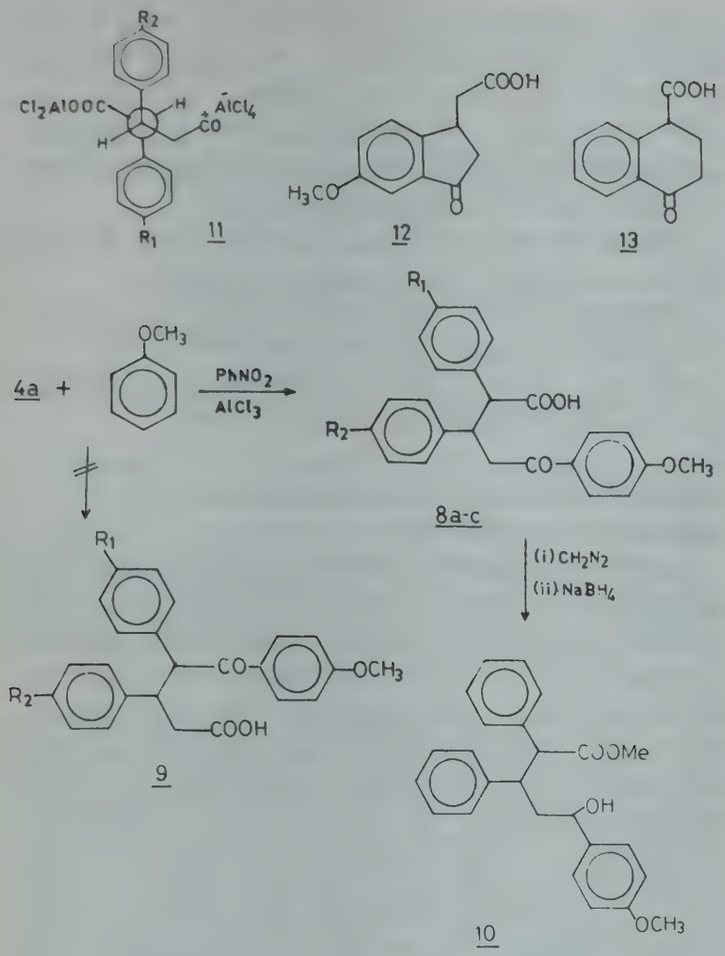


Table 1—^{13}CNMR Data of **7a**, **12** and **13**

[Chemical shifts in δ, ppm]

Compd	C-1	C-2	C-3	C-α	Other signals
7a	204.6	41.55	41.16	55.58	173.68, 122.88, 126.53, 127.65, 128.00, 128.81, 134.14, 137.28, 138.07, 155,47
12	204.6	43.43	39.36	33.39	173.13, 104.59, 123.06, 126.63, 137.68, 149.73, 159.13
13	197.59	43.91	25.21	35.06	177.79, 127.27, 127.93, 129.18, 131.80, 133.66, 139.61



ring for cyclisation. It may be assumed that the activation energy favours this type of cyclisation. Thus, stereochemistry seemed to govern the course of the reaction as against the electronic demand. The mechanism also rationalises the formation of a tetralone from 2-aryl[4] or 2,2-diarylpentanedioic anhydride[5] under similar conditions.

When a mixture of **4** and anisole in nitrobenzene was treated with $AlCl_3$, **8** was obtained and not the isomeric compound **9**. Benzene, however, failed to condense with **4**, contrary to the previous observation[2]. When a mixture of **4** and $AlCl_3$ in nitrobenzene was stirred at room temperature and anisole was added to it, **7** was obtained as the sole product. It showed the kinetic preference for the indanone formation. To determine the position of the anisoyl part in **8**, the product was esterified with CH_2N_2 and the resulting methyl ester reduced with $NaBH_4$ to the corresponding alcohol (**10**). The PMR spectrum of **10** exhibited a triplet at δ 4.15 due to $>CH$-OH indicating the presence of CH_2 group adjacent to it, and thus supported the structure of **8** as 2,3-diaryl-5-(4-methoxyphenyl)-5-oxopentanoic acid.

All the melting points are uncorrected. IR spectra were recorded on a Perkin Elmer 137 spectrophotometer (ν_{max} in cm^{-1}), PMR spectra on a Varian A-60D spectrometer and ^{13}CNMR spectra on a Jeol instrument using TMS as internal standard in both the cases (chemical shift in δ, ppm).

2,3-Diphenylpentanedioic acid (**3a**) was prepared according to the reported procedure[1] and converted into the anhydride **4a** by treatment with Ac_2O.

2-(4-Methoxyphenyl)-3-phenylpentanedioic anhydride (**4b**)

Ethyl 4-methoxyphenylacetate (19.4 g, 0.1 mol) was added to sodium ethoxide solution in ethanol (2.3 g Na in 10 ml ethanol) and the mixture refluxed for 5 min, cooled to room temperature and ethyl cinnamate (17.6 g, 0.1 mol) added to it. The resultant solution was refluxed for 4 hr, cooled, acidified with dil. HCl and kept overnight in a refrigerator. The diester of **3b** was obtained as a white solid which was crystallised from aq. ethanol, m.p. 63-64°, yield 30.7 g (83%). The diester was refluxed with KOH solution (20%, 20 ml) in aq. ethanol (50%) for 6 hr, the mixture cooled and washed with ether, and the aq. layer acidified with 50% HCl. The diacid **3b** thus obtained was crystallised from aq. ethanol (50%), m.p. 172-73°, yield 26.6 g (85%) (Found: C, 68.7; H, 5.8. $C_{18}H_{18}O_5$ requires C, 68.8; H, 5.7%).

The dicarboxylic acid **3b** was refluxed with Ac_2O (10 ml) for 4 hr. The solution on cooling deposited crystals of **4b** which were washed with ether-pet. ether (70:30), m.p. 127-28°, yield 90%.

3-(4-Methoxyphenyl)-2-phenylpentanedioic acid (**3c**) was prepared following the above procedure using ethyl 4-methoxycinnamate and ethyl phenylacetate, m.p. 224-25°, yield 78% (Found: C, 68.9; H, 5.8. $C_{18}H_{18}O_5$ requires C, 68.8; H, 5.7%). The acid **3c** on treatment with Ac_2O gave **4c**, m.p. 163-64°, yield 87%.

Reaction of **4a** *with* $AlCl_3$

To a well stirred solution of **4a** (2.66 g, 0.01 mol) in nitrobenzene (20 ml) was aded slowly $AlCl_3$ (3.9 g, 0.03 mol). The solution was stirred at room temperature for 5 hr, ice-HCl added to it, steam-distilled and the residue treated with aq. $NaHCO_3$. The bicarbonate solution was filtered, decolourised, washed with ether and acidified with HCl. The regenerated acid (**7a**) was crystallised from ethanol, m.p. 154-55°; yield 1.86 g (70%); 2,4-DNP: m.p. 261-62° (d), M^+ 266 (Found: C, 76.7; H, 5.3. $C_{17}H_{14}O_3$ requires C, 76.7; H, 5.3%); IR (KBr): 1280 (Ar−O−Me), 1680 br (−CO− and −COOH), 3300-3600 (−OH); PMR (CF_3COOH): δ 2.53 (1H, *dd*, J_{gem} = 20 Hz, J_{vic} = 4 Hz, 2-H_A), 2.53 (1H, *dd*, J_{gem} = 20 Hz, J_{vic} = 7.5 Hz, 2-H_B), 3.58 (1H, *d*, J = 10.5 Hz, α-CH), 4.06 (1H, *m*, 3-H), 6.4-7.7 (9H, *m*, Ar−H), 5.5 (1H, *bs*, −COOH).

4b and **4c** were treated similarly with $AlCl_3$ in nitrobenzene to obtain **7b** and **7c** respectively. Their characterization data are as follows:

7b: m.p. 145-46°, yield 72% (Found: C, 72.9; H, 5.48. $C_{18}H_{16}O_4$ requires C, 73.0; H, 5.4%); IR (KBr): 1280 (Ar − O − CH_3), 1670 (CO), 1710 (−COOH), 3300-3600 (−OH); PMR ($CDCl_3$-DMSO-d_6): δ 2.57 (1H, *dd*, J_{gem} = 20 Hz, J_{vic} = 3 Hz, 2-H_A), 3.04 (1H, *dd*, J_{gem} = 20 Hz, J_{vic} = 6 Hz, 2-H_B), 3.53 (1H, *d*, *J* = 10 Hz, α-CH), 3.83 (3H, *s*, Ar − OCH_3), 4.0-4.23 (1H, *m*, 3-H), 5.5 (1H, *bs*, COOH), 6.5-7.6 (8H, *m*, Ar − *H*).

7c: m.p. 218-19°, yield 68% (Found: C, 72.9; H, 5.5. $C_{18}H_{16}O_4$ requires C, 73.0; H, 5.4%), IR (KBr): 1260 (Ar − O − CH_3), 1665 (CO), 1695 (−COOH), 3400-3600 (−OH); PMR (CF_3COOH): δ 2.83 (1H, *dd*, J_{gem} = 20 Hz, J_{vic} = 3.5 Hz, 2-H_A), 3.37 (1H, *dd*, J_{gem} = 20 Hz, J_{vic} = 6.5 Hz, 2-H_B), 3.8 (1H, *d*, *J* = 10 Hz, α-CH), 3.93 (3H, *s*, Ar − O − CH_3), 4.07-4.53 (1H, *m*, 3-H), 6.5 (1H, *d*, *J* = 9 Hz, 4-H), 7.1 (1H, *dd*, *J* 4,5 = 9 Hz, *J* 5,7 = 2.4 Hz, 5-H), 7.4 (6H, *s*, C_6H_5 and 7-H), 10.28 (1H, *s*, −COOH).

When a mixture of compound **4** and $AlCl_3$ was stirred in nitrobenzene at room temperature and anisole added to it, compound **7** was obtained as a sole product.

Reaction of **4a** *with anisole in the presence of* $AlCl_3$

To a well stirred solution of **4a** (1.33 g, 0.005 mol) and anisole (0.6 ml) in nitrobezene (20 ml), was added $AlCl_3$ (2 g) gradually. The mixture was stirred at room temperature for 5 hr, poured into ice-HCl, the solution steam-distilled, and the residue dissolved in $NaHCO_3$ solution. The solution was filtered, washed with ether, decolourised and acidified with HCl. The crude compond **8a**, thus obtained, was crystallised from ethanol, m.p. 190-91°, yield 1.30 g (70%) (Found: C, 75.5; H, 5.9. $C_{24}H_{22}O_4$ requires C, 77.0; H, 5.9%), IR (KBr): 1260 (Ar − O − CH_3), 1674 (Ar − CO − CH_2), 1700 (COOH), 2900-3200 (−OH); PMR ($CDCl_3$ + DMSO-d_6): δ 3.48 (2H, *d*, *J* = 7.2 Hz, Ar − CO − CH_2), 3.85 (3H, *s*, −OCH_3), 3.98 (1H, *d*, *J* = 7.2 Hz, Ph − C*H* − COOH), 4.2 (1H, *m*, Ph − C*H* − CH_2 −), 6.85 (2H, *d*, *J* = 8 Hz, protons *ortho* to OCH_3), 7.85 (2H, *d*, J = 8 Hz, protons *meta* to OCH_3), 6.85-7.45 (10H, *m*, Ar − *H*).

8b and **8c** were prepared similarly from **4b** and **4c**, respectively, by the reaction with anisole.

8b: m.p. 230-31°, yield 65% (Found: C, 74.2; H, 5.9. $C_{25}H_{24}O_5$ requires C, 74.3; H, 5.9%).

8c: m.p. 156-57°, yield 70% (Found: C, 74.3; H, 6.0. $C_{25}H_{24}O_5$ requires C, 74.3; H, 5.9%).

Methyl 5-hydroxy-5-(4-methoxyphenyl)-2,3-diphenylpentanoate (**10**)

An ethereal solution of **8a** was treated with diazomethane in ether to obtain its methyl ester. The methyl ester (0.75 g) was stirred with $NaBH_4$ (1 g) in CH_3OH for 4 hr. The mixture was poured in water and the product extracted with ether. Removal of ether gave **10** as a thick oil which was purified by column chromatography over silica gel using benzene as eluant; IR (neat): 1742 (−COOMe), 3100-3540 (−OH); PMR ($CDCl_3$): δ 1.6 (1H, *bs*, −OH), 2.2-2.4 (2H, *m*, −CH_2−), 2.92-3.18 (1H, *m*, Ph − C*H* − CH_2 −), 3.74 (1H, *d*, *J* = 4 Hz, −CH − COOMe), 3.72 (3H, *s*, −$COOCH_3$), 3.87 (3H, *s*, −OCH_3), 4.32 (1H, *t*, *J* = 8 Hz, C*H* − OH), 6.86-7.3 (14H, *m*, Ar − *H*).

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A Dithienylacetylene Ester from *Eclipta erecta* Linn.†

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A dithienylacetylene ester, isolated from the roots of *Eclipta erecta* Linn., has been characterized as 5′-senecioyloxymethylene-2-(4-isovaleryloxybut-3-ynyl)dithiophene (V) on the basis of spectroscopic studies. Several known mono-, di- and ter-thienyl derivatives have also been isolated and their identities established either by direct comparison with authentic samples or by extensive spin decoupling experiments.

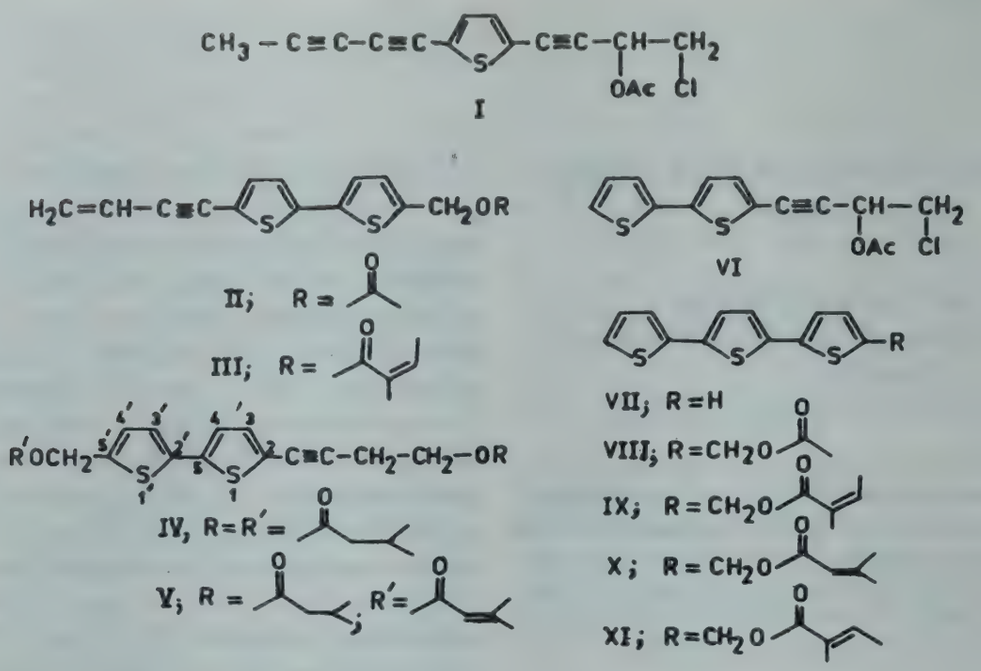
In continuation of our phytochemical survey[1] of Indian Compositae, we have reinvestigated the roots and the aerial parts of *Eclipta erecta* Linn. (tribe, Ecliptinae), an endemic plant of Jaipur region. Previous work on this plant led to the isolation of a number of thiophene derivatives[2,3]. The occurrence of mono- di- and tri-thiopheneacetylenes together with terthienyl in this species is remarkable.

The air-dried aerial parts (2 kg) and roots (500 g) of *E. erecta* collected from Jaipur, were extracted separately with pet. ether-ether (2:1) mixture for 24 hr in cold and the extracts were concentrated under reduced pressure. Concentrated extracts were column chromatographed over silica gel (60-120 mesh) and following fractions were collected in each case. Fraction-1 (pet. ether), fraction-2 (pet. ether-ether, 4:1), fraction-3 (pet. ether-ether, 1:1), fraction-4 (ether, 100%) and fraction-5 (ether-methanol, 4:1). Fractions-1,3,4,5 of aerial parts yielded negligible amount and hence not worked up. However, fraction-2 was found to be a mixture of four compounds. This fraction was subjected to preparative TLC (aluminium sheets 0.1 mm; SiO_2 PF 254; detected by UV light and by spraying with $KMnO_4$ solution). Elution with pet. ether-ether (9:1) afforded 2-angeloyloxy-5′-(but-3-en-1-ynyl)dithiophene (III) (10 mg, R_f 0.5), 5′-isovaleryloxymethylene-2-(4-isovaleryloxy but-3-ynyl)dithiophene (IV) (15 mg; R_f 0.45), stigmasterol (50 mg) and β-sitosterol (25 mg).

†Presented at 15th IUPAC International Symposium on the Chemistry of Natural Products, August 17-22, 1986, the Hague, the Netherlands.

In the case of roots again fractions 3,4 and 5 gave unworkable amounts. Fraction-1 gave a yellow crystalline solid (60 mg), m.p. 95°, which was identified as terthienyl(VII). Fraction-2 was found to be a complex mixture of ten compounds and after repeated separation by TLC employing pet. ether-ether (19:1, several developments) gave 2-angeloyloxymethylene-5′-(but-3-en-1-ynyl)dithiophene (III) (14 mg; R_f 0.85), angeloyloxymethylene terthienyl (IX) (10 mg; R_f 0.8), senecioyloxymethyleneterthienyl (X) (10 mg, R_f 0.7), 5′-senecioyloxymethylene-2-(4-isovaleryloxy but-3-ynyl)dithiophene (V) (10 mg, R_f 0.69), tigloyloxymethylene terthienyl (XI) (6 mg, R_f 0.65), 2-acetoxymethylene-5′-(but-3-en-1-ynyl)dithiophene (II) (12 mg; R_f 0.6), 2-(3′-acetoxy-4′-chlorobut-1-ynyl)-5-(pent-1,3-diynyl)thiophene (VI) (5 mg; R_f 0.57), acetoxymethyleneterthienyl (VIII) (5 mg; R_f 0.5) and 5′-isovaleryloxymethylene-2-(4-isovaleryloxybut-3-ynl)dithiophene(IV) (7 mg; R_f 0.48).

The new dithiophene ester (V), was obtained as yellow oil and its structure was established mainly by UV and high resolution PMR spectra. Its molecular formula $C_{23}H_{26}S_2O_4$ was followed from accurate mass measurements of its abundant ion peak at 328.0623 $[M-C_4H_9COOH]^+$ corresponding to molecular composition $C_{18}H_{16}S_2O_2$. It displayed a broad UV maximum at 335 nm characteristic of such dithiophene chromophoric systems and was comparable with the literature[4,5]. Its PMR spectrum was suggestive of the presence of an isovalerate and a senecioate residues. Two distinct doublets of three protons each at δ 2.19 and 1.89 with allylic coupling (J= 1 Hz, each) indicated the presence of two olefinic methyls of senecioyloxy group. A quartet of quartet at δ 5.79 (J= 1, 1 Hz) was assigned to the olefinic proton of the ester. A sharp, two-proton singlet at δ 5.27 was assigned to a methylene group bridged between the senecioate moiety and the thiophene ring. The presence of an isovaleryloxy function was established by the appearance of a six-proton doublet at δ 0.93 (J= 7 Hz, $2\times CH_3$), a two-proton methylene doublet at 2.3 (J= 7 Hz) and a one-proton methine multiplet at 2.1. A pair of triplets of two protons each at δ 2.78 and 4.25 was assigned to $C\equiv C-CH_2-$ and $-CH_2-O-$ methylene protons respectively. Signals in the aromatic region at δ 7.01 (*d*), 7.00 (*d*), 6.98 (*d*) and 6.97 (*d*) (J= 3.5 Hz, each) were assigned to H-3′, H-3, H-4′ and H-4 thiophenic protons respect-



ively. The sequence of protons was established by spin decoupling. The relative position of ester moieties could be deduced by comparing the chemical shifts of methylene groups attached to these moieties with those of corresponding diacetate from *Porophyllum*[5] and diisovalerate earlier isolated from this plant[3].

In its mass spectrum the molecular ion peak was not very prominent. However, an abundant ion peak at *m/z* 328 was attributed to $[M-C_4H_9COOH]^+$ corresponding to the fragment $C_{18}H_{16}S_2O_2$ as confirmed by its high resolution spectrum at *m/z* 328.0623. The other important fragments were at *m/z* 330 $[M-C_4H_7COH]^+$, and 227 $[328-C_4H_9COOH]^+$ which also corroborated the presence of two esters in the molecule.

The identity of the known compounds was established by direct comparison with authentic samples or by spin decoupling of their PMR spectra.

The isolation of IV and V again supports the close morphological relationship of *Eclipta* to *Porophyllum*, *Dyssodia* and *Tagetes*.

Biogenetically these thiophenes are probably formed from acetylenic precursors. This is evident from the fact that mono- and dithiophenes still have acetylenic bonds. In some cases feeding experiments with labelled precursors have shown that they are formed in the plants from a widespread tridecapentaynene, a C_{13}-acetylene[6]. The first step is the addition of H_2S or its biochemical equivalent to conjugated triple bonds, to form a thiophene ring.

The biogenetic route of dithiophene involves the addition of two equivalent of H_2S to tridecapentaynene. Feeding experiments have established this assumption for several dithiophenes[6] which followed enzymatic oxidation to gave different bithienyl derivatives.

The IR spectra (ν max in cm^{-1}) were recorded in CCl_4/KBr on Perkin-Elmer models 137E and 337 and PMR spectra in $CDCl_3$ on a Bruker WM400 (400 MHz) instrument (chemical shifts in δ-scale downfield from internal TMS) and the UV spectra on Perkin-Elmer model 202 automatic recording spectrometer and Beckmann model DU in ether or in ethanol (95%). Mass spectra were recorded on a Varian MAT 711 at 70 eV.

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Conformational Studies on (L-Glutamic Acid)$_n$-hexamethylenediamine-(L-Glutamic Acid)$_n$

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The effect of *p*H on the conformations of hexamethylenediamine bound oligomers of L-glutamic acid is reported.

Homo- and hetro-polypeptides have intensively been studied inorder to understand the nature of folding of the backbone of protein molecules and also to establish a correlation between structure and their specific biological action[1]. The conformation of oligo-polypeptide depends on the nature of the amino acid side chain, chain-length and degree of ionisation. Glutamic acid oligomers exhibit a variety of conformations depending on the chain-length and *p*H value. The conformations of the glutamic acid oligomers prepared by initiation of primary amine have been studied in detail by Rinando *et al.*[2] In this note the effect of *p*H on the conformations of hexamethylenediamine bound oligomers of glutamic acid has been reported. These systems can be considered as models for the broken helices which appear in the intermediate state of the helix-coil transition and in the folding of α-helices in protein molecules.

The required hexamethylenediamine bound γ-benzyl-L-glutamate oligomers were synthesised as described earlier[3]. Three systems with $n=8$, 16 and 30 were deblocked by HBr/AcOH (36% W/W).

Circular dichroism (CD)

Circular dichroism spectra of the oligopeptides were recorded on a Jasco-500A spectropolarimeter at *p*H 2.25 and 6.6 using solutions of about 1 mg/1 ml concentration and cell of 1 mm path length.

CD spectra of the three systems with $n=8$, 16and 30 at *p*H 2.25 a (clear two units below the *p*K value of the γ-carboxyl group) are shown in Fig. 1.All the systems exhibited n-η ≒ and $\pi-\pi^*$ bands. The n-π^* band for $n=8$ system underwent a hypsochromic shift to 200 nm. The ellipticity for 205 nm band observed for the system with $n=30$ was 13.4 deg cm^2 d mol^{-1}.

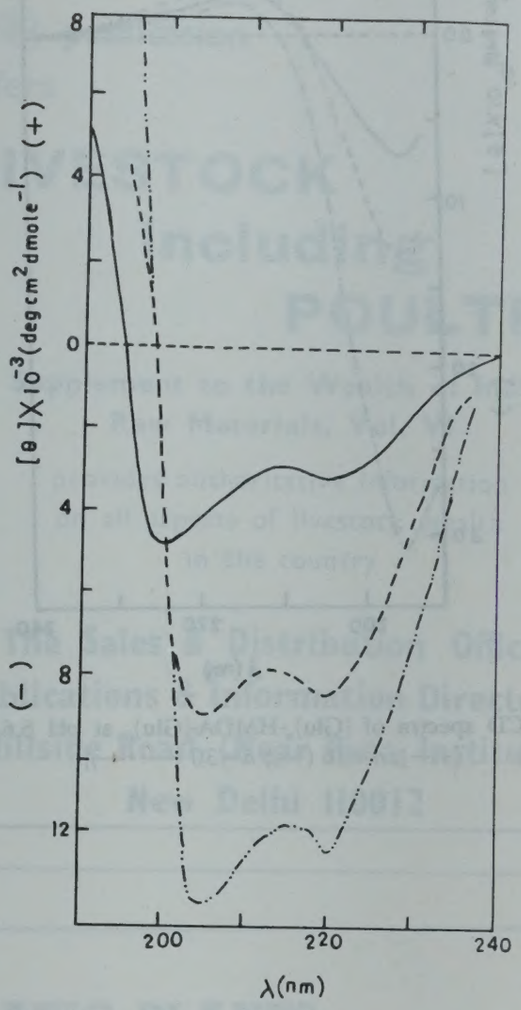

Fig. 1—CD spectra of (Glu)$_n$ at *p*H 2.25 [$n=8$(—); $n=16$ (---); $n=30$ (---·---)]

The CD spectra (Fig. 2) of the three systems at 6.6 *p*H where the γ-carboxyl is completely ionised were characterised by a negative minimum around 195 nm and a small positive maximum at 215 nm. The ellipticity of the 195 nm band again increased with the increase in chain-length but the intensity of the 215 nm band remained more or less the same. These features were attributed to the circular dichroism of random coil conformation where there is no correlation between ψ and Φ angles of neighbouring peptidic residues.

A comparison of the Rinando data with the present bifunctionally polymerised systems (Table 1) revealed some significant features. When $\alpha=0$, the ellipticity of the uniterrupted helices is about three times that of the once interrrupted helices, (G)$_n$HMDA(G)$_n$ suggesting that the major role is played by hexamethylene moiety in disurpting the formation of helix with adjacent residues when the chains are fully charged. The ellipticity of the po-

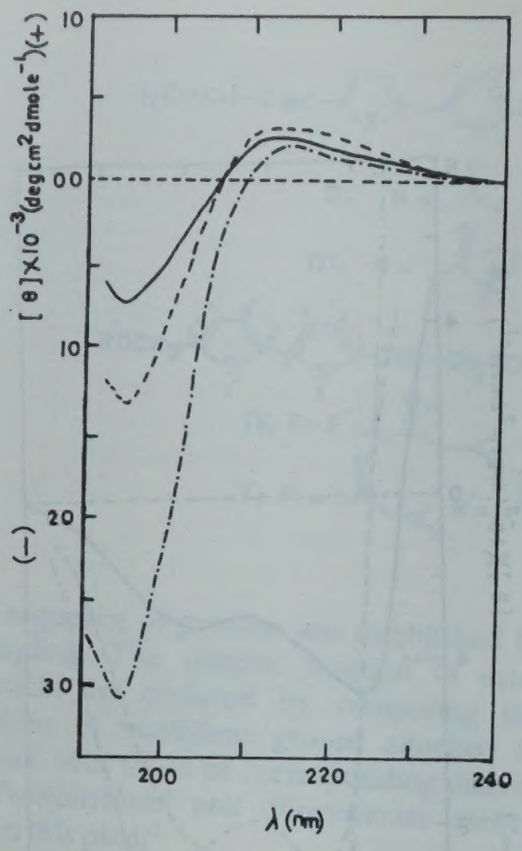

Fig. 2—CD spectra of $(Glu)_n$-HMDA-$(Glu)_n$ at *p*H 6.6 [$n=8$ (——); $n=16$ (---); $n=30$ (---·---)]

Table 1—Ellipticities of $(Glu)_n$-HMDA-$(Glu)_n$ in deg cm^2 d mol^{-1} × 10^{-3}

n	$\alpha=0$	$\alpha=1$
2×8	4.8	9.5
16*	15.0	22.0
2×16	8.7	12.0
27*	26.0	26.4

*Rinando's data

lymers under study are relatively higher when $\alpha=1$ in comparison with uninterrupted chains, suggesting that the constraints imposed by hexamethylene chain on the conformational angles are apparently less when the glutamyl side chains are charged.

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